THE IMPACT OF WHISKY BLEND MATRICES ON THE SENSORY PERCEPTION OF PEATY FLAVOURS

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CHAPTER 1. INTRODUCTION

1.1 Scotch whisky

The success of Scotch whisky in the global drinks market is well-known. One of the reasons for this is its unique sensory properties (Lyons 1999). The majority of Scotch whiskies are produced and sold as blended whisky (a blend of different types of whiskies, see Chapter 1.1.3). Thus blended whisky is one of the most popular spirits in the world. The creation of Scotch blends is regarded as an art and every Scotch whisky Company has its own proprietary recipes, particularly for some of the more established brands. Some blenders believe that during whisky blending certain types of aromas may be suppressed or enhanced by other aromas in the blend, a phenomenon recognised in other areas of the food and drink industry (Booth et al. 1989). However the aroma interaction between different congeners and the resulting sensory experience is poorly understood. For master blenders, it also becomes more and more difficult to handle the fast growing industry requirement of product consistency and other organoleptic requirements by traditional working ways. This introduction describes the factors that contribute to the sensory characteristics of Scotch whisky, before addressing the physiological attributes of humans that allow them to perceive such characteristics.

1.1.1 History of Scotch whisky

Whisky has been produced in Scotland for hundreds of years. The first recorded mention of distilling in Scotland was in the 4th and 5th centuries and that was introduced by the Irish monks (Bathgate 2003). During the 16th century, the dissolution of the monasteries resulted in the spread of distilling knowledge from the monks to the other spirit manufacturers (Morrice 1983).

The whisky that came from these distilleries was made primarily from malted barley which had been kiln-dried over peat fires. A greater volume of grain whiskies was produced by some processes such as the one invented in earlier 19th century by Robert Stein and further enhanced by Aeneas Coffey (Campbell 2003a). Blending was

pioneered by Andrew Usher, in Edinburgh, in the early 1860s (Conner et al. 2003). Prior to the introduction of blending, Scotch whisky only had a limited appeal. It was hypothesized that the traditional single malt whiskies available at that time were perhaps too strongly aromaed for everyday consumption. However, in the late 19th century the phylloxera epidemic destroyed most of the vineyards for wine grapes in Europe, most notably in France, so failure of grape crop (e.g. Cognac), and high taxes on wine and gin in England provided the business opportunity for blended whisky to enter the English market. Together, with the introduction of milder blended whiskies, the appeal of Scotch whisky spread firstly to England and gradually expanded all over the world. Since the early 1900s, Scotch whisky has become the best-selling spirit drink in the world (Bathgate 2003). In the late 1970s, around 99% of malt whisky was used for blending (Craig 1994; Gray 2012), today blended Scotch whisky constitutes about 90% of the whisky produced in Scotland. The blending of whiskies put Scotch on to the world stage (Conner et al. 2003).

1.1.2 Definition of Scotch whisky

A Royal Commission in 1909 ruled that "whisky" is a word that can only be used to describe spirit obtained by distillation from a wash saccharified by the diastase of malt, and that "Scotch whisky" is whisky produced according to this definition under Scottish regulation. The production of Scotch whisky is regulated by the UK Scotch whisky Act 1988, the Scotch whisky Order 1990 (Scotch whisky Act 1988) and, under the designation 'whisky', by the EU Spirit Drinks Regulation no.1576/89 (Halliday 2004). The most recent Scotch whisky regulation (2009) were issued by Scotch whisky Association on 2/12/2009, They replaced the Scotch whisky Act 1988 and the Scotch whisky Order 1990 (Scotch-Whisky-Regulations 2009).

1.1.3 Whisky classification by categories

Scotch whisky is classified into two main types, malt and grain whisky (Scotch Whisky Regulations 2009). These whiskies differ based on their nature and the proportion of cereals used as raw materials as well as the type of distillation used in the production (Takefumi 2007). From these, three categories of blended whiskies can be produced; blended malts, blended grains and blended Scotch whisky.

A single malt Scotch whisky is a product of one specific distillery and has not been mixed with whisky from any other distilleries. Single malt Scotch whisky is characterised by malted barley which is the only cereal used in the manufacture. The so-called pot still is used for the distillation of malt whisky. Heat is then applied either directly or indirectly to the pot containing the alcohol liquor or wash. This is known as batch distillation. A blended malt is a mix of malt whiskies produced from different distilleries (Scotch-Whisky-Regulations 2009). A Single grain whisky is a product of one grain distillery and is usually made from wheat, corn or unmalted barley. There are seven grain distilleries in Scotland. The majority these are in central Scotland with the exception of Invergordon in the northern Highlands and Girvan in the far south. Continuous column stills are used for grain whisky production. The development of continuous distillation facilitated the production of a large quantity of grain whiskies at a lower cost per litre of alcohol than their batch distilled counterparts. A blended grain is a mix of grain whiskies produced from different distilleries (Scotch-Whisky-Regulations 2009). Blended Scotch whisky is defined under the SWR as a combination of one or more single malt Scotch whiskies with one or more Single Grain Scotch whiskies , which accords with traditional practice (Scotch-Whisky-Regulations 2009). The introduction of blending as a means of reducing the strength of aroma and the cost of whisky was therefore the first systematic attempt to control the aromas of the product.

1.1.4 Whisky classification by region

Scotch whisky production was originally divided into two regions, the Highlands and the Lowlands, for tax purposes under the Wash Acts (1784). The ‘Highland line’ labelled in Figure 1.1 transverses Scotland from the east coast to the west, which is a straight line crossing from Dundee in the east to Greenock in the west (Figure 1.1).

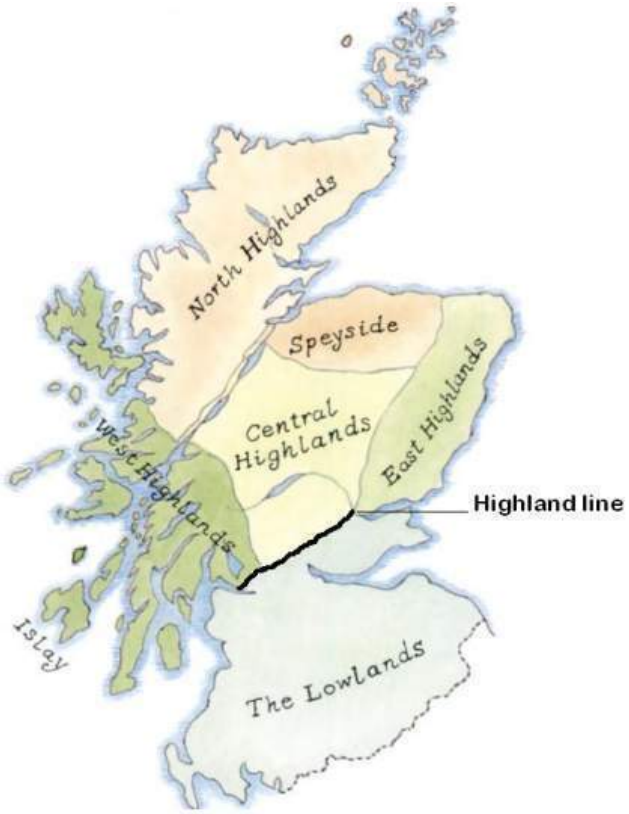


Figure 1.1 – Map of Scotch whisky production regions (Wishart 2006).

More recent geographical classifications divide Scotch malt whiskies into the 'Highlands', the 'Lowlands', 'Islay' and 'Campbeltown whiskies' (Simpson et al. 1974), or 'Highlands', 'Greater Speyside', 'Lowlands' and 'Islay'. The 'Highlands' whiskies can be further sub-divided into 'Northern', 'Central-Southern', 'Eastern', 'Western' and 'Island' (MacLean 1997; Wishart 2006) as being shown in Figure 1.1. Until 1900, the region of origin made a discernible contribution to the whisky aroma, but over time these geographical influences have been reduced. One exception to this are the Islay whiskies, which still have distinctive peat characters (Canaway et al. 1984). Otherwise the geographical split is difficult to justify nowadays due to the number of distilleries currently operating in each region (Highland (30), Lowlands (3), Islay (9) and Speyside (48). It is therefore found difficult to avoid the conclusion that the major importance for geographical classification is to locate a distillery on the map.

1.1.5 Whisky classification by aroma

An alternative approach is to classify Scotch whiskies based on their aroma characteristics. Sensory perception of whisky products tends to be more meaningful to consumer expectation than mere geographical grouping.

Table 1.1 – Classification of single malt whiskies by aroma (Wishart 2006).

Cluster	Aroma Characters	Distilleries
A	full-bodied, medium-sweet, pronounced sherry with fruity, spicy, malty notes and nutty, smoky hints	Balmenach, Dailuaine, Dalmore, Glendronach, Macallan, Mortlach, Royal Lochnagar.
B	medium-bodied, medium-sweet, with nutty, malty, floral, honey and fruity notes	Aberfeldy, Aberlour, Ben Nevis, Benrinnes, Benromach, Blair Athol, Cragganmore, Edradour, Glenfarclas, Glenturret, Knockando, Longmorn, Scapa, Strathisla
C	medium-bodied, medium-sweet, with fruity, floral, honey, malty notes and spicy hints	Balvenie, Benriach, Dalwhinnie, Glendullan, Glen Elgin, Glenlivet, Glen Ord, Linkwood, Royal Brackla.
D	light, medium-sweet, low or no peat, with fruity, floral, malty notes and nutty hints	An Cnoc, Auchentoshan, Aultmore, Cardhu, Glengoyne, Glen Grant, Mannochmore, Speyside, Tamdhu, Tobermory
E	light, medium-sweet, low peat, with floral, malty notes and fruity, spicy, honey hints	Bladnoch, Bunnahabhain, Glenallachie, Glenkinchie, Glenlossie, Glen Moray, Inchgower, Inchmurrin, Tomintoul
F	medium-bodied, medium-sweet, low peat, malty notes and sherry, honey, spicy hints	Ardmore, Auchroisk, Bushmills, Deanston, Glen Deveron, Glen Keith, Glenrothes, Old Fettercairn, Tomatin, Tormore, Tullibardine
G	medium-bodied, sweet, low peat and floral notes	Arran, Dufftown, Glenfiddich, Glen Spey, Miltonduff, Speyburn
H	medium-bodied, medium-sweet, with smoky, fruity, spicy notes and floral, nutty hints	Balblair, Craigellachie, Glen Garioch, Glenmorangie, Oban, Old Pulteney, Strathmill, Tamnavulin, Teaninich
I	medium-light, dry, with smoky, spicy, honey notes and nutty, floral hints	Bowmore, Bruichladdich, Glen Scotia, Highland Park, Isle of Jura, Springbank
J	full-bodied, dry, pungent, peaty and medicinal, with spicy, feinty notes	Ardbeg, Caol Ila, Lagavulin, Laphroaig, Talisker

Table 1.1 shows an example of single malt whisky classification by aroma character which was derived by Wishart (2006). In his study, 86 single malt (10 to 15 year old) whiskies were evaluated. A classification of single malt whisky was focused, using

benchmark malt whiskies sampled from each distillery. Twelve standard aroma attributes were selected: body, sweetness, smoky, medicinal, tobacco, honey, spicy, winey, nutty, malty, fruity and floral, were used as parameters for scoring of each whisky. The resultant profiles were collated to classify the tested malt whiskies by using cluster analysis. The malt whiskies were categorized into 10 groups (A to J in Table 1.1) based on the similar scores for all twelve aroma characteristics (Wishart 2006). Although, this study has many limitations, it is a very interesting new approach to classify the Malt Scotch whisky by their aroma characteristics.

1.2 Origins of Scotch whisky aroma

The aroma of alcoholic beverages is generated from many different volatile organic compounds. These aroma and aroma compounds give the spirit its typical odour and taste. Nowadays, nearly one thousand compounds have been identified in different beverages (Lehtonen and Jounela-Eriksson 1983) and more compounds are continually being discovered as analytical developments continue. Whiskies constitute a complex mixture of hundreds of aroma compounds in an ethanol–water matrices (Nykänen and Suomalainen 1983a). The aroma and aroma compounds in Scotch whisky originate from the various raw materials and production stages, including kilning, fermentation, distillation, maturation and blending, with each of these elements playing their own specific role in aroma and aroma formation(Suomalainen and Nykanent 1970; Suomalainen and Lehtonen 1976; Paterson and Piggott 1989).

In this section, each key processing step will be considered in terms of its impact on Scotch whisky aroma. It will give a general context for this study and support the interpretation for following chapters. The following subChapters briefly describe the aroma contribution of each stage in Scotch whisky production.

1.2.1 Raw materials

1.2.1.1 Air and Water

Ideally, all distilling operations are carried out in an environment without any air pollution. In malting, it is particularly important that the air used in the germination

and the kilning must meet the relevant hygiene requirements, in order to ensure the high and consistent quality of malt and to prevent the formation of any unwanted compounds, such as volatile nitrosamines (Dolan 2003).

The quality of water plays an important role in the production of malt whisky. Water is used for various purposes, including malting, mashing, fermentation and the reduction of alcoholic strength (to cask and bottle strength). In addition, certain standards must also be followed when using water for whisky production. All potable water samples are required to follow such standards, i.e. they must be microbiologically and physically clean and as pure as possible (Halliday 2004).

1.2.1.2 Cereals in whisky production

Barley

In the manufacture of Scotch malt whisky, malted barley is employed as a source of enzymes (principally amylolytic) that catalyse the hydrolysis of starches. According to the legal definition of Scotch whisky: Distillation of a mash made from malted cereals with or without whole grains of other cereals, which has been: saccharified by the diastase of the malt contained therein, with or without other natural enzymes. This also includes the grain whisky process (Bringhurst et al. 2003).

Maize

Maize was traditionally recognized as a prime cereal used for Scotch grain whisky (Bringhurst et al. 2003). Corn is a popular grain in whisky production because of its high content of starch. The starch is readily gelatinized and is converted into fermentable sugars to give a higher yield of spirit. Nowadays in Scotland, it has been largely displaced by European wheat, owing to the price effects of the EU agricultural policies (Conner and Piggott 2003).

Wheat

Since 1984, wheat has predominately been used as the grain source for grain whisky production in Scotland (Bringhurst et al. 2003). Soft winter wheat (*Triticum aestivum*) is used due to its relatively high starch content and low protein content (Bringhurst et al. 2003).

1.2.2 Malt kilning

When the required enzyme content and the degree of modification have been reached, the resultant green malt (malted barley) is kilned to give a dry product suitable for storage and to cease further development of any biological activities (Bathgate and Cook 1989).

Malt kilning is one of the major aroma development stages. During kilning, many new compounds are formed and others, which exist in the green malt, are removed. These chemical changes affect the sensory quality of the final distilled spirit. The chemistry of the formation of aroma compounds during kilning is complex, and the levels of such compounds become higher as the kilning temperatures increases (Griffiths 1992). The formation of these aroma components in malt is mainly by three different routes:

1. Enzymatic and chemical oxidation
2. Maillard reaction
3. Peating

1.2.2.1 Enzymatic and chemical oxidation

Most volatiles characterized in unprocessed barley are formed by enzymatic and chemical oxidation of unsaturated fatty acids (Campbell 2003b). During kilning, the predominant fatty acid in barley lipids is linoleic acid (C18:2) which is transformed into 9- and 13-hydroperoxides by lipoxygenases I and II respectively, and then further converted into aldehydes, ketones, alcohols, and acids. Some of these were identified as possessing grainy or green cereal-type odours (Tressl et al. 1983; Paterson and Piggott 1989).

1.2.2.2 The Maillard reaction

A range of important aroma compounds (cooked cereal, corn-like and bready aromas) are formed by the Maillard reaction, in which free amino acids and sugars combine and undergo chemical transformation such as Strecker degradation, which lead to the production of unsaturated aldehydes, furans and pyrroles etc. (Paterson and Piggott

1989). These aroma components formed the during whisky malt kilning are not as important as in brewery malt kilning, due to the distilling malt being lightly kilned compared to, say, pale lager malt for beer production. This helps to ensure that the enzymatic activity of the malt is as high as possible for activity during mashing and fermentation(Tressl et al. 1983; Bathgate and Cook 1989). Indeed at least one grain distillery derives its diastatic power from unkilned, so-called green, malt as this provides significantly higher levels of enzyme activity that conventional distillers' malt.

1.2.2.3 Peating

The smoke of peat fires gave the malt a distinctive tang allowing the Scottish product to be instantly identifiable by whisky drinkers all over the world (Bathgate 2003). Peat is decayed plant material that has formed over thousands of years, and is generally found in the wetland areas (Shotyk 1988). The chemical composition of peat is derived from a combination of mire plants and microorganisms, and also varies based on the qualities of soil and water (Williams and Yavitt 2003). During kilning, the peat is burned without flaming and smoke is produced called peat reek. Any aroma-active volatile compounds in peat smoke are then introduced into the airflow during the kilning processes(Shotyk 1988; Bathgate and Cook 1989). Peat is burned during the early stages of kilning, so that a portion of the combustion products adsorb to and are absorbed by the malt.

Phenolic compounds derived from peating process

It is now generally accepted that the intensity of adsorbed peat smoke on malt is roughly proportional to the detected levels of phenolic substances, such as phenol, cresols, eugenol, and guaiacol. Nowadays, the measurement of phenol content in peated malts has become the accepted measure of the degree of peating (Table 1.2; (Paterson and Piggott 1989; Bathgate and Taylor 1997). During spirit production, only a small proportion of the phenols (about 4%), find their way into the cask (Howie and Swan 1984). A large proportion is lost through the pot ale and spent lees with only minimal loss via the draff, as phenols have relatively high boiling point and high solubility in water phase than the solid phase.

Table 1.2 – The relative intensity of peating generally indicated by phenol level in new make spirit (Bronsky and Schumann 1989; Dolan 2003).

Peaty level	Total phenols (mg/L)
Lightly peated	1-5
Medium peated	5-15
Heavily peated	15-50

Many peat smoke-derived aroma compounds have previously been reported in peated malt, ranging from simple hydrocarbons to complex heterocyclic compounds (Deki and Yoshimura 1974). These include a range of phenolic compounds, carbohydrate-derived compounds and nitrogen-containing compounds. It has been shown that 'smoky' is not an individual characteristic but is comprised of various smoky odour/aroma (Chambers et al. 1998).

It is certainly true that phenolic compounds are the most distinctive 'marker' compounds in peated malt (Piggott et al. 1996). Nevertheless, they are not the only constituents of peat smoke, as peat smoke contains a wide range of aroma compounds. More than eighty aroma components have been reported in peated malt derived from peat smoke and while phenols have been implicated in peaty aroma, it is still not certain that the phenolic constituents are the principal contributors to the characterized aroma of peated malt whiskies (Deki and Yoshimura 1974). It has also been noted that kilning is not the only phenol source in whisky production. There are other sources of phenolic compounds produced during mashing, fermentation, maturation and possibly introduced by water (Steinke and Paulson 1964; Jounela-Eriksson and Lehtonen 1981; Paterson and Piggott 1989; Beek and Priest 2000).

Non-phenolic peat-related compounds

Volatile non-phenolic compounds contained in smoke have an influence on aroma in smoked food (Steinke and Paulson 1964; Fiddler et al. 1966; Jounela-Eriksson and Lehtonen 1981; Sterckx et al. 2011). It has been reported in studies on peat smoke pyrolysis that aromatic compounds, including alkylbenzenes, naphthalenes, benzofurans and acetophenone have been found, which may contribute to the aroma of peated malts (Shafizadeh 1982; Harrison and Priest 2009; Schellekens et al. 2009). Compounds such as furfural and 5-HMF have previously been identified in peated

malts. Compounds in this class are known to possess caramel and burnt aroma notes and so could contribute to the aroma of peated malt (Maga 1988; Chambers et al. 1998; Harrison and Priest 2009). Five classes of nitrogen compound have also been detected in whisky at low levels: aliphatic amines, thiazoles, pyrazines, pyridines and quinolones (MacFarlane et al. 1973; Heide 1986; Withers et al. 1996).

1.2.3 Fermentation

Fermentation is regarded as a key stage of the formation of aroma compounds in whisky (Lehtonen and Jounela-Eriksson 1983; Korhola et al. 1989). The main aromas generated due to the action of yeast during fermentation are higher alcohols, fatty acids and esters. These compounds are found in relatively high concentration in the final product. To date, investigations have shown that the aroma fractions of the various alcoholic beverages are qualitatively similar, because of the typical appearance of the same components. Yeast largely produces the same aroma compounds irrespective of sugar source (Suomalainen and Lehtonen 1976). Although yeast produces qualitatively much the same aroma compounds, the quantitative composition can vary greatly even in the same type of beverage (Lehtonen and Jounela-Eriksson 1983).

The nature and quantity of the compounds formed in the fermentations are greatly affected by the fermentation conditions, such as temperature, pressure, Oxygen supply, yeast type and yeast nutrients (Lehtonen 1983b). In Scotch whisky production, the absence of wort boiling allows bacteria to survive and to contaminate the mash. Lactic acid bacteria are the most predominant bacteria found in the wort fermentation (Geddes and Riffkin 1989). This is due to their ability to utilise sugars such as pentoses (not readily utilised by yeast). They grow well at pH 4 – 5 and are able to tolerate high concentrations of ethanol i.e. 10 – 12% (v/v); (Korhola et al. 1989). Bacterial growth normally increases rapidly at the end of fermentation resulting from the metabolism of yeast autolysis products (Berry 1984; Simpson et al. 2001). Bacterial fermentation can make a positive contribution to the quality of the spirit and aroma characteristics (Dolan 1976; Dolan 1979; Geddes and Riffkin 1989; Harrison and Priest 2009). The following groups of compounds are largely produced by yeast metabolism and are considered to have significant contribution to aroma in Scotch

malt whisky (Jounela-Eriksson 1978).

1.2.3.1 Higher alcohols

Higher alcohols are formed by the catabolism of glucose and amino acids in the wort. Isobutanol, propanol, 2-methylbutanol, 3-methylbutanol and 2-phenylethanol are formed initially during the exponential phase of yeast growth and are produced continuously in the linear phase of fermentation (Christoph and Bauer-Christoph 2007). 2-Methylbutanol and 3-methylbutanol, the mixture of which is also called isoamyl alcohols, is the most abundant of the "minor" components of the distilled spirits that are synthesised by yeasts. Depending on the nature of the raw material, these alcohols comprise of 40 – 70% of the total fusel alcohol fraction (Suomalainen and Lehtonen 1979).

From an aroma perspective, the threshold values for the fusel alcohols are rather high and therefore they tend not to contribute significantly to the aroma characteristics in the final whisky products. The term ‘fusel alcohols’ refers to their malty and wort characters (Beal and Mottram 1994), with the exception of 2-phenylethanol, which has a rose-like fragrant floral odour (Nykänen and Suomalainen 1983b).

1.2.3.2 Esters

Esters are the largest group from aroma intensity perspective of aroma compounds in Scotch whiskies. Their quantities and relative proportions are of the great importance for the perceived aroma since their concentrations are generally above the sensory threshold values (Nykänen and Suomalainen 1983a). The esters in Scotch whiskies can be divided into three main groups according to their retention time in the gas chromatograph.

Light fraction

The first group is the so-called light fraction, comprising mainly of ethyl acetate, isobutyl and 2- and 3-methylbutyl. This group generally has relatively low aroma threshold levels and generally exhibits pleasant aromas, perceived as fruit and solvent like, the so-called ‘fruit esters’ (Suomalainen and Lehtonen 1979; Nishimura and

Matsuyama 1989; Christoph and Bauer-Christoph 2007). Ethyl acetate is the most abundant ester in Scotch whiskies. It accounts for about 80% (by concentration) of the total esters in the whicky final product and is produced by a reaction between ethanol and acetate, during fermentation it is a reaction between acetyl coenzyme A and ethanol. Bacterial contamination during fermentation of the wash results in high levels of acetic acid in the wash which reacts with ethyl alcohols to produce higher levels of ethyl acetate in the product.

Middle fraction

The middle ester fraction contains the compounds from ethyl hexanoate (C6), octanoate (C8), decanoate (C10), and dodecanoate (C12). As the chain length increases the notes change from ethyl hexanoate which is fruity-like, to ethyl dodecanoate which has a soapy and oily character (Suomalainen and Lehtonen 1979).

Heavy fraction

The third group is heavy fraction consisting of compounds with the lowest volatility. It is composed of C14-C18 fatty acid esters. These esters as well as the long-chain fatty alcohols may contribute to the waxy- and oily-like aroma which is the characteristic of some Scotch malt whiskies (Christoph and Bauer-Christoph 2007).

1.2.3.3 Acids

The biosynthesis of acids produced during alcoholic fermentation is initiated in the yeast cell by the formation of acetyl coenzyme A, which reacts with malonyl coenzyme A to form mainly saturated straight-chained fatty acids (Christoph and Bauer-Christoph 2007). The volatile fatty acids contribute to the aroma of fermented beverages like wine or beer and their concentrations are often high. Acetic acid is the most common acid component, typically making up about half of the total volatile acids in Scotch whisky. After acetic acid the most abundant acids are octanoic (C8), decanoic (C10) and dodecanoic acids (C12), the majority of which come from fermentation (Suomalainen and Lehtonen 1979; Lehtonen and Jounela-Eriksson 1983).

1.2.4 Distillation - separation and fractionation of aroma

1.2.4.1 Malt Distillation

Distillation is used as an effective separation process and is regarded as the basis of the production of high-alcohol beverages. Distillation separates mixtures based on their volatilities. In whisky distilleries, more volatile components (i.e. ethanol) are separated from less volatile components (i.e. water), by condensing and collecting the alcohol-rich vapours released from boiling aqueous alcohol.

The distilling pot stills were traditionally made of copper. It remains the only suitable material for distilling high quality malt whisky at the present time (Nicol 2003). Although its mode of action was unsuspected at the outset of whisky production, copper takes part in a number of chemical reactions, which greatly affect the aroma of the spirit (Conner and Piggott 2003). A pot still (Figure 1.2) is thought to influence final spirit composition due to its structural design, although such effects are still unequivocally demonstrated. The variations in the pot shape such as, swan neck and lyne arm, that affect the degree of reflux and can potentially affect the composition of the new make spirit (Nicol 1989; Nicol 2003). Changing the degree of reflux within the still can affect the degree of contact between vapour and still copper surface, with consequent variations in the spirit profile.

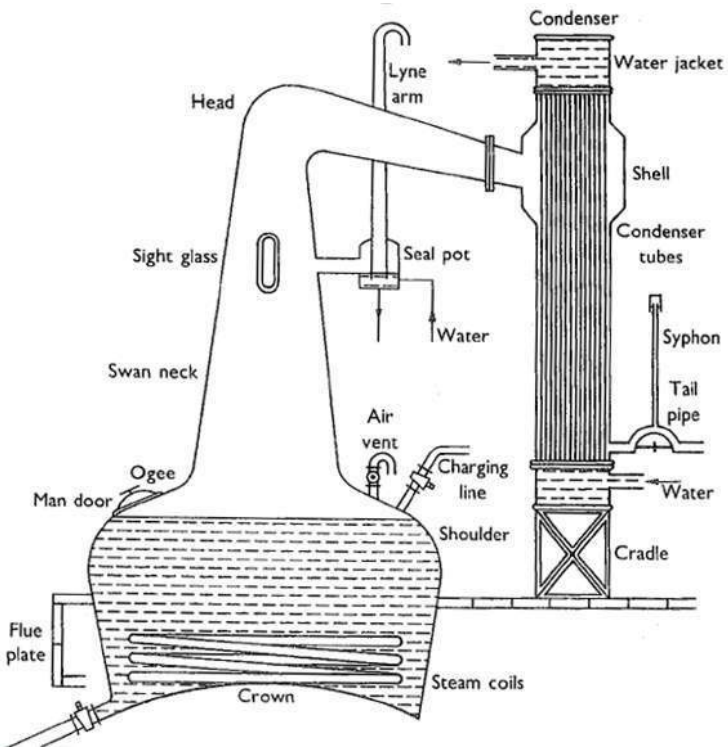


Figure 1.2 – Plain pot still (Nicol 1989).

In malt whisky production, distillation not only functions as a means to recover volatiles from fermented wort (or wash), but can also stimulate further, potentially aroma-relevant reactions due to the distillation temperatures achieved. There are many factors that influence these interactions, but most are related to the level of the contact with the copper of the still, such as esterification (Watson 1983b). The effect of copper on the concentrations of congeners is shown in Table 1.3. Copper can also catalyse the reduction of aldehydes to alcohols. The recycling of the foreshots and the feints enhances these reactions, converting acids and aldehydes into more desirable congeners such as alcohols and esters (Watson 1983b; Nicol 1989). The presence of the copper also reduces the level of sulphur compounds in the final spirit (Nicol 2003). All of these copper-mediated reactions explain the differences in spirit quality due to the differences in still design or operations, which result in varying level of copper contact, either through physical shape, rate of distillation or levels of reflux (Watson 1983a; Paterson and Piggott 1989; Nicol 2003; Harrison et al. 2011).

Table 1.3 – Congener levels (g/100 L alcohol) in spirit produced in the laboratory glass still apparatus, with and without the introduction of copper (Watson 1983b).

Esters	No Copper	Copper	percentage increased
Ethyl acetate	9.67	14.58	51%
Isoamyl acetate	0.23	0.74	222%
Ethyl octanoate	0.22	0.34	55%
Ethyl decanoate	0.07	0.25	257%
Isoamyl decanoate	0.09	0.15	67%
Ethyl dodecanoate	0.32	0.52	63%
Ethyl tetradecanoate	0.16	0.26	63%
Ethyl hexadecanoate	0.30	0.55	83%
Phenyl ethyl acetate	0.69	1.12	62%

1.2.4.2 Grain distillation

In the early 19th century, Aeneas Coffey invented the Coffey still, which was developed based on the original design by Robert Stein a few years earlier, and even today it is still the most efficient and the cheapest in terms of raw materials, labour and energy cost (Panek and Boucher 1989; Lyons 1999; Piggott 2003). To this day the processes of continuous cooking, mashing and distillation are routinely applied in

grain distilleries.

In the production of Scotch grain whisky, the Coffey still is constructed with two columns which are positioned side-by-side. The first 'analyser' column separates the alcohol from the wash, while the second column, known as the 'rectifier', is designed to remove any unwanted aroma compounds from the final spirit, also that the wash is preheated by passing through a copper tube running through the rectifier (Campbell 2003a). Figure 1.3 shows a simple diagram of a Coffey still.

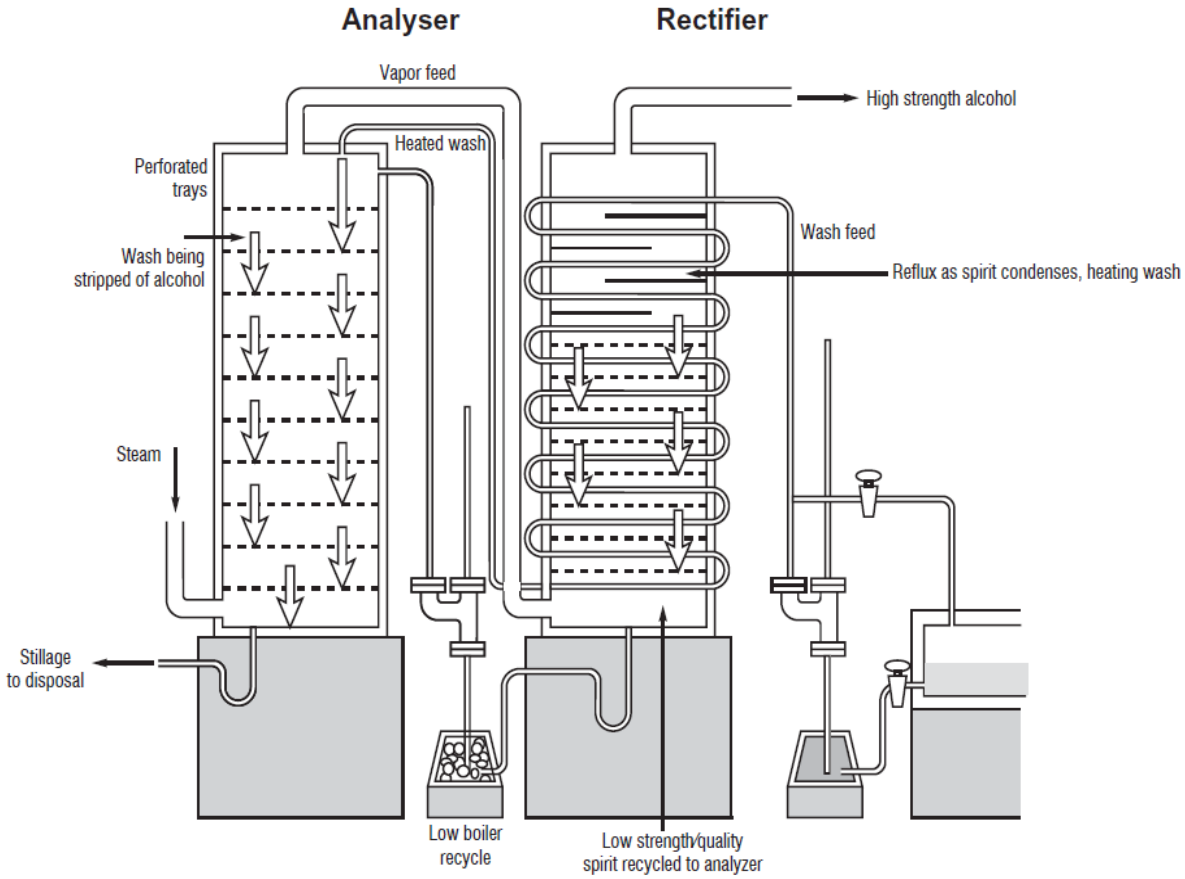


Figure 1.3 – Essential features of the Coffey still (Piggott 2003).

One of the great advantages of the Coffey still is that the temperature gradient in the rectifier creates different liquid profiles across the rectifier column. By selecting the plate at which spirit is drawn off, the congener present in the final spirit can be controlled (Figure 1.4).

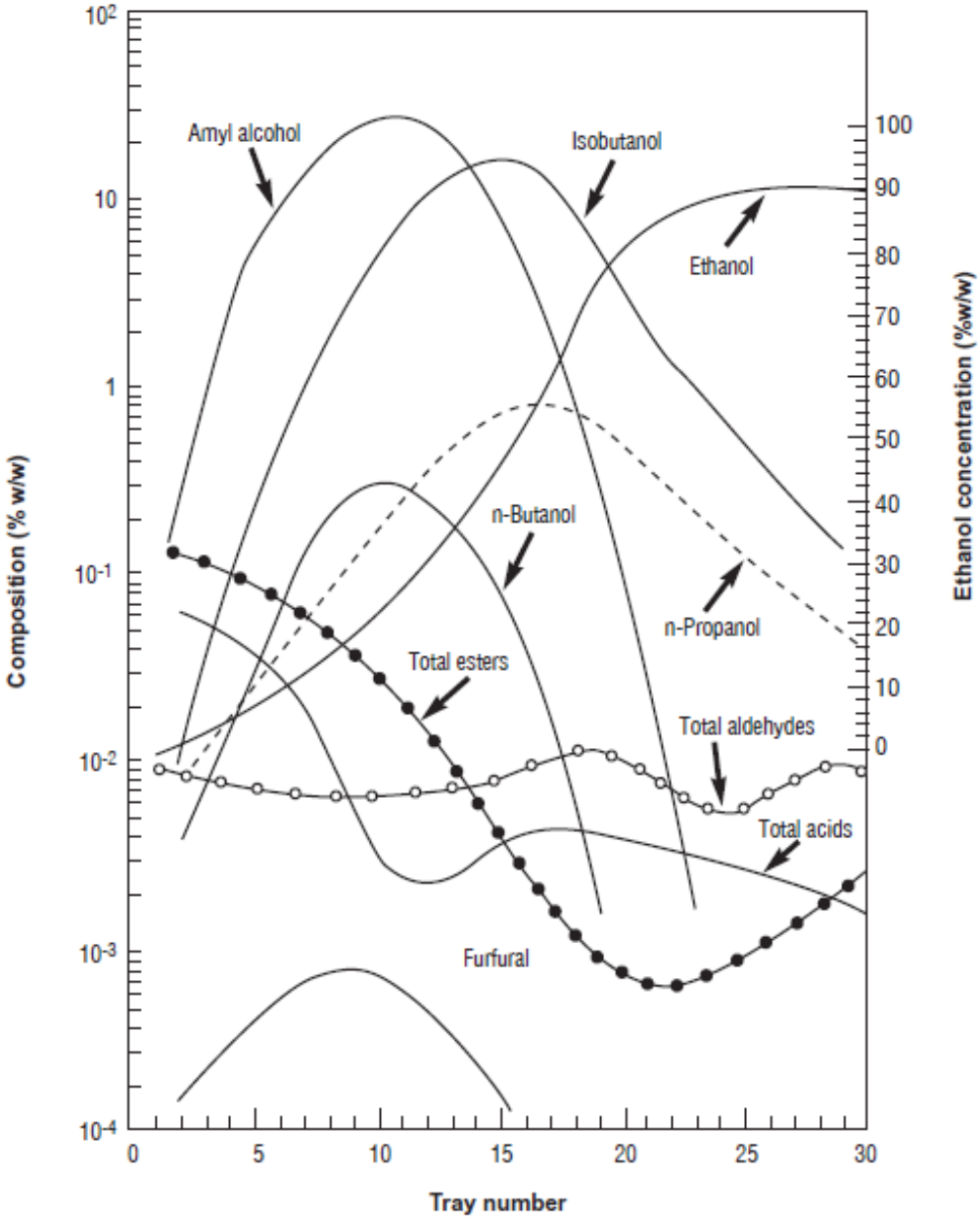


Figure 1.4 – Typical concentrations of compounds in the rectifier (Dolan 1976).

For example, the stream containing high levels of iso-amyl alcohol is separated from ethanol and collected from near the base of the column. Iso-amyl alcohol is recycled and sold mainly to the perfume industry. The highly volatile compounds, which concentrate towards the top of the column, are collected as vapour. These include ethanol, which is condensed and fed back to the still, and some sulphur compounds, which are vented to the atmosphere (Bringhurst et al. 2003; Campbell 2003a).

The concentrations of aliphatic alcohols in distilled spirits vary over a wide range and depend mainly on the type of distillation, separation, and fractionation. There are no

remarkable differences between the new make spirit and the matured whisky on the basis of higher alcohol content (Nykänen and Suomalainen 1983b).

Nevertheless, there is a major analytical difference of the higher alcohol profiles between the Scotch grain and malt whiskies. Grain whiskies have relatively small amounts of amyl alcohol, since most of the higher-boiling congeners are removed from the spirits during continuous distillation (Nykänen and Suomalainen 1983a; Aylott et al. 1994). For comparison, the levels of propanol, isobutanol, 2-methylbutanol, 3-methylbutanol and 2-phenylethanol in different types of Scotch whiskies are summarised in Table 1.4.

Table 1.4 – Contents (mg/100 ml of pure alcohol) of higher alcohols in whiskies (Nykänen and Suomalainen 1983b).

Scotch Whiskies	Propanol	Isobutanol	2 and 3-Methylbutanol	2-Phenylethanol
Malt	42-54	82-122	183-220	4.4-6.2
Grain	56-70	48-81	4-17	0-0.9
Blend	39-73	50-93	62-94	1.2-3.1
Low-price*	28-72	34-72	20-61	0.6-2.1

** Low-priced commercial blended Scotch whiskies usually contain lower levels of malt whisky compared to their higher priced counterparts*

1.2.5 Maturation

Fresh made spirits usually have pungent, unpleasant odours and sharp tastes (Conner et al. 2003). Major changes - in aroma terms - occur in the chemical composition of the new make spirit in a cask during its maturation period. After maturation, the pungent and feinty aromas of the new distillate transform into the typical mellow characteristics of a mature whisky, and the colour of the spirit changes from colourless clear to golden brown. The legal minimum maturation time for Scotch whisky is three years, a restriction that also applies to grain whiskies used in blends. However, there is no simple relationship between maturation time and the quality of the final product. Low levels of wood aromas may positively enhance distillate characteristics, whereas prolonged maturation may give strong wood aromas that mask distillate characters (Conner et al. 2003). The major cask types used in the Scotch whisky industry are ex-bourbon and ex-sherry casks, which have previously

been used in bourbon and sherry maturation, respectively.

In the Scotch whisky industry, first-fill oak casks (the used cask from bourbon or sherry industries), have a rapid initial wood extract extraction period during the first six to 12 months. During this period, any free extractives are rapidly diffused from the cask wood to the spirit. For refill casks (been used for Scotch whisky maturation more than once), most of the free extractives have been depleted in the previous whisky maturation. Therefore, there is no initial rapid extraction for the maturation in refill casks and the overall extraction rate is apparently lower than the maturation in first-fill casks. The decline in the extraction implies a decrease in the development of mature characteristics, such as woody vanilla and sweet character (Conner et al. 2001; Conner et al. 2003). Consequently, the cask will eventually fail to produce a sufficiently matured spirit, even after many years of maturation. Therefore, some treatments such as re-charring and rejuvenation have been applied to solve this problem. These treatments can yield similar aroma compounds to those produced in a new charred cask. However, some constituents of oak are not regenerated, such as oak lactones and hydrolysable tannins. Consequently, the balance of wood extractives in regenerated casks is different from that in a new charred cask (Conner and Piggott 2003). Maturation is an essential processing step which improves the aroma since fresh distillates often have unpleasant odours and tastes. During the maturation period, it is clear that the cask is more than just a physical container for the spirit. A range of physical and chemical interactions take place between the barrel, the surrounding atmosphere and the maturing spirit which transform both the aroma and the composition of the spirit. The time required for satisfactory maturation varies according to the storage parameters: new make spirit characteristics, cask size, wood treatment and particularly the type of barrel used. The change in the aroma of the maturing spirits is due to the changes in the composition and the concentration of the compounds influencing the taste and aroma. These changes may be caused by:

1. Extraction of wood components
2. Chemical interaction
3. Adsorption and evaporation.

1.2.5.1 Extraction of wood components

Extractives are compounds found in oak wood that are soluble in either water or organic solvents. These compounds are believed to be important in the maturation of whisky and are classified by the properties of colour and volatility. They can be broadly classified into colour, volatiles and non-volatiles.

Colour

The development of colour is most rapid during the early stages of maturation. The rate of colour development decreases each year. As the maturation proceeds in the first fill cask, the colour changes from colourless to light to deep yellow, then to amber and finally to reddish yellow .

Volatile compounds

The extraction and subsequent transformation of the compounds from the oak cask are believed to be of the prime importance to the final aroma. The degradation of lignin to aromatics occurs during the charring or toasting of oak, allowing for the development of compounds available for slow extraction, such as vanillin, coniferaldehyde, sinapaldehyde and syringaldehyde (Reazin 1983b; Conner et al. 2003). Of these, vanillin is of the greatest sensory importance on account of its low odour threshold. Some volatile phenols are formed (Reazin 1983a; Conner et al. 2003) during high temperature toasting and charring, such as guaiacol and syringol (Conner et al. 1993; Conner et al. 2003). Many studies have found increased levels of the aromatic aldehydes, i.e. vanillin and syringaldehyde, by the application of charring treatments and increased levels of other aldehydes and esters . Whisky lactones (*cis*-β-methyl-γ-octalactone and *trans*-β-methyl-γ-octalactone) were found in unheated treated wood (Withers et al. 1995; Mosedale and Puech 1998). These whisky lactones are the important components, contributing to the aroma with low threshold values and appear to have different sensory characters at different concentrations (Salo et al. 1972; Mosedale and Puech 1998; Piggott 2003).

Non-volatile compounds

Semi-volatile and non-volatile compounds of wood change the colour of the distillate and contribute to a properly matured aroma (Conner et al. 2003). There are a number

of non-volatile chemical classes extracted from oak during the maturation, such as tannins, organic acids, sugars, as well as glycerol (Nishimura and Matsuyama 1989; Mosedale 1995; Mosedale and Puech 1998). These compounds are extracted rapidly during the first six months of maturation, followed by a slow linear increase with time (Figure 1.5) (Nishimura and Matsuyama 1989).

1.2.5.2 Chemical interactions

Maturation in oak barrels is also accompanied by changes in the chemical composition of the whisky. These changes are attributable to the oxidation of components present in the original whisky as well as those extracted from the wood (Grajski and Freeman 1989).

Distillate congener reactions

Different components of a fresh distillate may react during the maturation period, which is favoured by high ethanol content and the presence of oxygen. The levels of acids, esters, and aldehydes increase during the maturation process. Aldehydes and esters increase roughly linearly throughout the maturation period, whilst the acid content increases mostly during the first year (Figure 1.5).

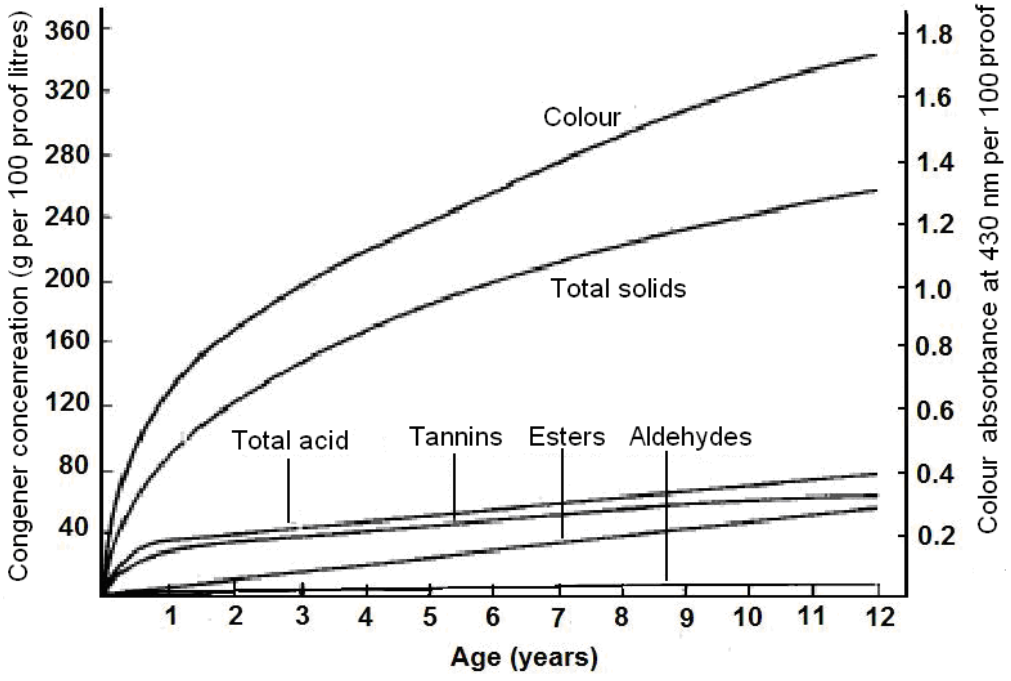


Figure 1.5 – Congener changes during maturation (Nishimura and Matsuyama 1989) 100 proof = 50% abv.

The increase in these compounds is largely due to the oxidation and the interaction of ethanol. In fresh made spirit, the concentration of fatty acids is significantly lower owing to the esterification and their separation by distillation. However, acetic acid can be produced during maturation through the oxidation of ethanol. Fresh made Scotch grain whiskies contain very little acids, but the acid content rises gradually during maturation (Suomalainen and Nykanent 1970; Lehtonen and Jounela-Eriksson 1983). The chemical mechanisms for these observations are summarized as follows (Reazin 1983a; Nishimura and Matsuyama 1989).

Reactions involving distillate and wood extract

Some of the compounds extracted from the oak will react with the distillate to create new congeners. For example, the lignin-derived aromatic aldehydes may be subjected to ethanolysis and esterification to produce esters and acids, due to interaction between aldehydes extracted from the cask and the product of ethanol oxidation (Reazin 1983a; Conner and Piggott 2003).

The formation of the "active" carbon layer on the inner surface of the cask is the result of carbonization of the polymeric constituents. This layer contributes little in the way of colour or extractives to the maturing whisky. It does however, play an important role in the removal of immature character, particular in reduce the concentration of sulphur compounds (Philp 1986; Fujii et al. 1992). Also, the breakup of the wood structure near the surface may allow easier penetration by the spirit and increase the extraction of degradation components from sub-surface layers (Mosedale 1995).

1.2.5.3 Adsorption and evaporation

Adsorption

Charring of the casks produces a layer of highly active adsorbent which effectively removes many undesirable congeners by adsorption onto, and diffusion into, the wood during the "immaturity" stage (Reazin 1983a; Clyne et al. 1993). Studies have found that charring can increase the removal of sulphur compounds, as they are adsorbed by charred wood (Philp 1989).

Evaporation

Oak barrels are permeable, allowing both ethanol and water to evaporate, resulting in the volume loss. A decrease in the volume of the cask contents during the maturation period causes the aroma to become more intense, complex, and concentrated (Nishimura and Matsuyama 1989). The relative rates of the loss of water and of ethanol depends on the cask size, alcohol strength of filling, maturation conditions and time (Withers et al. 1995). In Scotland, where the whisky barrels are stored in cool and humid warehouses, the alcoholic strength decreases during the maturation. In contrast, American bourbon storage conditions cause an increase in alcoholic strength due to the relatively hot and dry weather condition (Nishimura and Matsuyama 1989; Conner et al. 2003).

As evaporation progresses, the level of spirit in the cask decreases that creates an air space ('headspace'). The increased headspace provides a larger volume of air to replenish the dissolved oxygen in the spirit that is used up in oxidation reactions during maturation. Evaporation of volatile compounds through the cask surface during maturation also occurs, which is thought to be one of the main route for the loss of undesirable sulphur compounds (Conner and Piggott 2003).

1.2.6 Whisky blending

Blending probably began in earnest in the 1860s when the firm of Ushers produced their "Old Vatted Glenlivet Whisky" by combining malt whiskies into a standard product. Even today blending is considered to be more of an art than a science (Conner et al. 2003). Because every distillery's whiskies have a character of their own the malt and grain whiskies must be chosen to complement and enhance their respective aromas. The formulation of a blend is not equivalent to following a fixed recipe. It is more dynamic, with the component whiskies and their addition levels varying from batch to batch.

If the primary aim of the blender is to produce a whisky of a definite and recognisable character, the second challenge is to achieve consistency. At the heart of this activity is the Master Blender. His, or her, responsibility is to ensure the consistency of the quality from batch to batch and to ensure that whisky of the appropriate quality and

age is available to achieve this. Consistent aroma, taste and overall quality are the key factors in a successful blend (Booth et al. 1989).

Generally, the approach in all whisky blending around the world is to use a light-bodied spirit (grain) as a base, and to mix with the added heavy-bodied aromaing spirits (malt) (Conner and Piggott 2003). During blending, component malts and grains are selected together that maybe mask, dilute, complement and enhance each other's aromas. The aroma interactions brought about by blending, have been little studied. Several studies have focused on perceptual interactions in binary odour mixtures, but few on more complex mixtures (Meilgaard 1975; Derby et al. 1991; Fritsch and Schieberle 2005; Poisson and Schieberle 2008). The aroma of whisky is an example of a complex odour mixture.

In theory, a blended whisky could be formulated using only a few malt whiskies. In practice, the number used is generally in a range of 20 to 50 malts (Booth et al. 1989). A large number of malts used in the blend allow maintaining the consistency of the blend quality. The spirit type available for blending can be summarized into the following four basic types, which are commonly used in blending practices in whisky production.

peated malt: Peaty aroma is widely recognized as the most valuable aroma character in Scotch whisky. In many blended products, peaty aroma plays an important role as a "signature". However, in most Scotch blends, the rich-aromaed peated malts are likely to be used in small quantities because high level of peated malted tends to be too dominant (Conner et al. 2003). Therefore, this study mainly concentrated on low level of peaty aromas which is a common level of peated malt used in whisky blends (based on the SWRI database).

Unpeated malt: Malt (distillation) gives heart character of the Scotch blends. Unpeated malt is mainly produced from the lowlands and Speyside, and is used as a major source to supply the key aromas and the complexity to whisky blend. It has less intense aroma characteristics. Unpeated malt has a range of aromas, as opposed to the dominant key notes found in peated malt. So, addition at higher levels will have a less distinctive sensory impact.

Grain whisky: Grain whisky was introduced into whisky blending practice for economic reason. The purpose of the introduction is to neutralize the heavy malt aroma character. However, grain whisky is not as simple as the cheap diluents, it has a significant role in blending, such as introducing smooth and sweet characters, rounding the characteristics of blend and revealing certain aromas in the malts (Booth et al. 1989; Conner et al. 2003).

Woody grain: Woody grain whisky is a type of grain whisky that imparts significant levels of woody aromas. Thus, it is strongly dependent on the maturation time and the history of the cask. Maturation is one of the most important steps to improve the whisky aroma. Considering an increased demand for deluxe blend products, strong woody character not only acts as an indispensable aroma element for whisky but also as a quality symbol. Therefore, whisky with strong woody character is commonly used in whisky blends to enhance the woody aroma (Conner et al. 2003).

It is in situations such as this that the blender must know the malt whisky sufficiently well to substitute the missing component with a replacement that is suitably compatible with the rest. In the interest of blend consistency, he/she must know the whiskies that are interchangeable and where they can be secured in the required quantities (Booth et al. 1989).

1.2.7 Whisky chill – filtration

For malt whiskies, haze might be formed due to the precipitation of long chain lipids and esters, being less soluble in water than in ethanol (Piggott et al. 1996). Chill filtration is, therefore carried out to remove cloud or hazes, which may be produced on dilution and chilling the bottle strength whisky. The filtration is undertaken at a temperature between 0 – 10°C by passing the whisky through sheets of cellulose. There is no substantial evidence to suggest that chill filtration significantly affects whisky composition or aroma, presumably as the components removed during the chill-filtration are mostly semi- or non-volatile compounds (Piggott et al. 1996).

1.3 The Olfactory System

1.3.1 Odorant Receptors

The sense of smell has long remained the most enigmatic of our senses. Odour receptors are located on the olfactory receptor cells, which occupy a small area in the upper part of the nasal epithelium and detect the inhaled odorant molecules (Figure 1.6). The olfactory epithelium contains millions of olfactory neurons, which send messages directly to the olfactory bulb of the brain (Axel and Buck 2004). The sense of smell in mammals is characterized by a range of physical and neural processes which begin with the olfactory receptor binding of odorous molecules, to transduction of chemical energy into electrical energy (Bell 1996; Bozza and Kauer 1998; Wang et al. 1998; Lin and Ngai 1999; Takefumi 2007). Olfactory receptors consist of protein chains (G-proteins) that penetrate the olfactory cell surface, traversing the cell membrane seven times, and is known as a seven transmembrane segment (7TMr). The chain creates a binding pocket to which the odorant can attach. All odorant receptors are related proteins and differ only in some amino acid residues. The subtle differences in the protein structure explain why the receptors are triggered by different odorant molecules. When the odorant molecules bind to receptors, the conformation of the receptor protein is altered, leading to G-protein activation, then the G-protein turn stimulate into the formation of cAMP (cyclic AMP). These messenger molecules activate the cell, opening the calcium ion channels and trigger a signal to be sent to the brain via nerve processes (Buck and Axel 1991; Laing and Jinks 1996; Axel and Buck 2004).

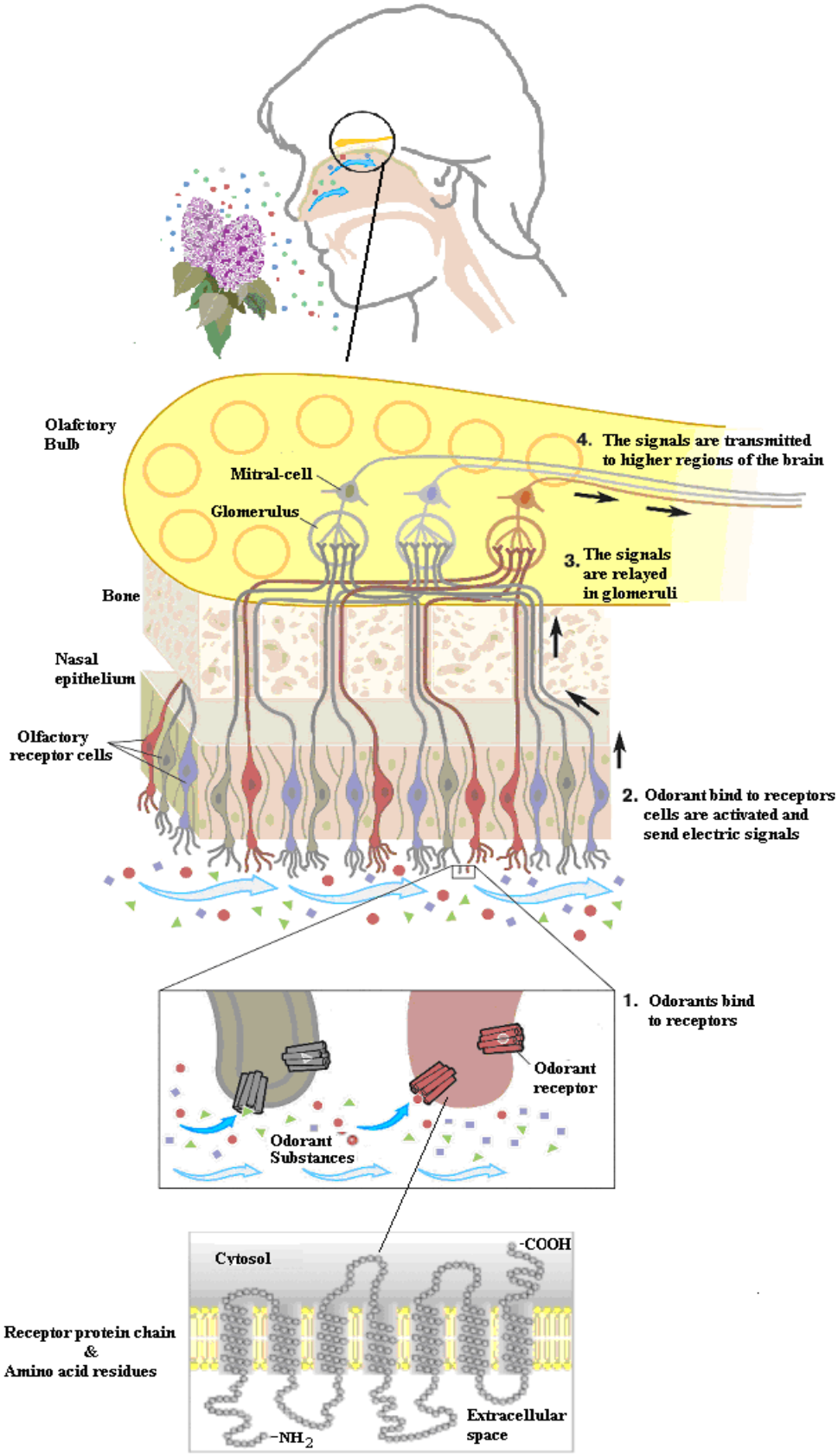


Figure 1.6 - Schematic illustration of the olfactory system (Axel and Buck 2004).

1.3.2 Olfactory Bulb

The olfactory receptors transduce the G-protein conformation change, by impulse conduction directly to distinct micro domains, called glomeruli, to the olfactory bulb, the primary olfactory area of the brain. Receptor cells of the same type are randomly distributed on the nasal mucosa but receptor cells carrying the same type of receptor concentrate their processes into the same glomerulus (Figure 1.6). From these glomeruli the information is relayed further to the mitral cells in the brain. Each mitral cell is activated by only one glomerulus, and the specificity in the information flow is thereby maintained. By way of long nerve processes, the mitral cells send the information to several parts of the brain. Most odours are composed of multiple odorant molecules, and each odorant molecule activates several odorant receptors. This leads to a combinatorial code forming an "odorant pattern". This is interpreted and leads to the conscious experience of a recognizable odour (Buck and Axel 1991; Bell 1996; Laing and Jinks 1996; Bozza and Kauer 1998; Axel and Buck 2004).

1.3.3 Complex Odour Mixtures

The odours we perceive in almost every instance of everyday life are derived from mixtures rather than due to single odorants. Very rarely do we perceive an odour that is produced by a single chemical. Thus the sense of smell may have developed to perceive and recognize odour mixtures rather than to sense the odour of a single chemical (Laing 1987). Actually, our olfactory system seems to rely on a combinatorial pattern to detect odorants and encode their unique identities. Different odorants are detected by different combinations of receptors and thus have a different pattern of receptor firing. These codes are translated by the brain into diverse odour perceptions (Figure 1.7). The immense number of potential receptor combinations is thought to be the basis for our ability to distinguish and form memories of more than 10,000 different odorants (Buck and Axel 1991; Laing 1994; Axel and Buck 2004).

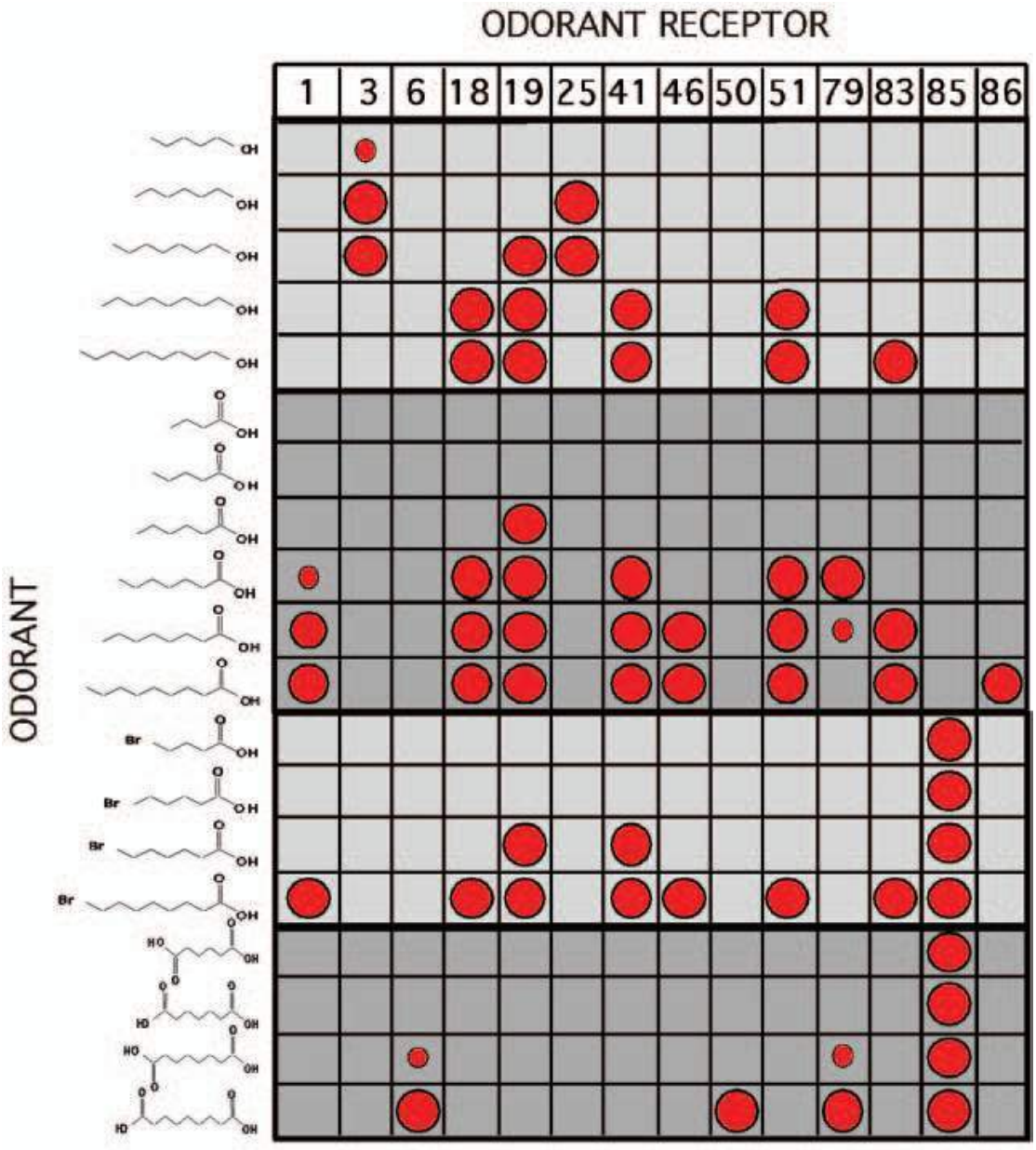


Figure 1.7 – Odorant receptors are used combinatorial to detect odorants and encode their identities (Malnic et al. 1999).

Complex stimuli, such as the smell of a rose, coffee, wine or fresh cut grass come to be perceived as unique unitary sensations, when in fact they consist of hundreds or even thousands of different active chemicals, most of which are capable of producing multiple sensations. Our olfactory system has the ability to simplify the enormous information from a complex odour, and uses a holistic approach to identify and memorize this as a resultant pattern, binding them together into a whole in a process known as unitization (McLaren et al. 1989; Livermore and Racher 2000). When odorants, with similar odour intensity, constantly present together under conditions

that promote unitization, they come to be bound together to create a unique pattern (spatial map) that is recognized as a single odour. While this pattern is initially variable and not reliable, with experience and repeated presentations, increasingly stronger patterns are formed, making up a stable unitary entity (Grajski and Freeman 1989; McLaren et al. 1989). This strong unitary pattern may then facilitate their recognition from a complex odorous background. However, if the odour matrices are overly complex the unitary pattern may not be identifiable, lowering of the original intensity through spatial and temporal filtering mechanisms see Chapter 1.4.2; (Laing and Francis 1989; Laing and Livermore 1992; Livermore and Racher 2000).

1.4 Aroma congener interactions

Aroma is defined as the combined perception of mouthfeel, taste, and aroma/odour. Mouthfeel and taste are perceived in the mouth. To be perceptible, taste compounds must be released into the saliva so that they can contact the gustatory cells of the buccal surface. Aroma compounds, on the other hand, will only be perceived if they are released into the headspace in the mouth and are carried to the olfactory epithelium in the nose (Taylor 1996; Baek et al. 1999; McClements 2004).

Despite intensive study, the relationship between the sensory whisky aroma attributes and the aroma compounds present in whisky has not yet been established (Jack 2003). In particular, there is a lack of understanding about the aroma interaction behaviour during whisky blending practice. Nevertheless, a quantitative understanding of this relationship is extremely difficult because of the complexity of the physicochemical, physiological and psychological processes involved (McClements 2004).

1.4.1 Physicochemical perspective

To perceive an aroma, the aroma compounds need to achieve a sufficiently high concentration in the vapour phase to stimulate the olfactory receptors. The key congener activity coefficients in whisky headspace may be influenced by the physicochemical properties of the liquid phase, such as temperature, pressure, wood extract and ethanol concentration, and interactions between aroma compounds and

non-volatile constituents in whisky (Williams and Rosser 1981; Conner et al. 1994; Conner et al. 1999b; Conner et al. 2001; Ebeler 2004). Therefore, the aroma perception of whisky aroma is not simply determined by the type and concentration of aroma molecules present, but also by their concentration in the headspace to stimulate the appropriate sensory receptors. For example, it was found that long chain fatty acid ethyl esters in whisky effectively trapped other aroma-active compounds in the liquid phase of the whisky preventing them from effectively contributing to the aroma (Conner et al. 1994; Conner et al. 1999a).

1.4.2 Physiological perspective

Aroma interactions at the physiological level are clearly complicated. Once the aroma molecules have reached the receptors they interact with them to produce electrochemical signals that are transmitted to the brain via the nervous system (as explained in Chapter 1.2.2). A great deal of research is being carried out to identify the molecular basis of aroma receptor interactions (Lawless 1986; Axel and Buck 2004). However, nowadays predicting the outcome of mixing odours is still a very difficult task. Current methods for predicting the importance of individual aroma attributes in whisky research are commonly based on threshold and concentration data and none incorporate information on the perceptual interactions of the constituents. Unfortunately, no procedure has been developed which can consistently predict the outcome of mixing odours.

In most human olfactory psychophysical studies of mixtures, the stimuli mainly consist of very simple odorant mixtures such as binary, ternary or quaternary mixtures (Laing and Willcox 1983; Jinks and Laing 1999). From these olfactory psychophysical studies, there are two important phenomena evident i.e. spatial and temporal filtering (Laing 1992).

Even with simple mixtures, humans can only discriminate and identify a limited number of odorants (Murphy 1987; Laing and Glmmarec 1992; Laing and Livermore 1992). Decreased odour similarity will enhance discrimination of the components within mixtures. Also identification of odorants in mixtures becomes more difficult as the number of odorants is increased (Jinks and Laing 1999). Human olfactory

perception appears to have a physiological limitation in their ability to discriminate and identify odours in mixtures.

Mixing odours results in partial or complete suppression of the perceived intensity of one or more components (Laing and Willcox 1983). Very few instances of odour enhancement have been reported. Most of these studies have been confined to combinations of odorants which individually were at sub-threshold concentrations (Rosen et al. 1962; Laska et al. 1990). With current methodology and instrumentation there is still great difficulty in predicting the perceptual outcome of a simple odour mixture. Clearly, it will be even more difficult to predict the important constituents of complex aromas such as blended whisky.

From previous studies, it is clear that there is substantial loss of odorant information when odours are mixed. Two mechanisms, namely spatial and temporal filtering, are proposed to account for this loss and to provide a basis for understanding and investigating the phenomenon of blending (Laing 1992).

1.4.2.1 Spatial filtering

When an odorant reaches the olfactory receptor epithelium it generally stimulates a substantial number of receptor cells. Accordingly, when two odours are present, they will compete for some of the receptor cells. Then the response pattern for both odours may be changed through competition for receptor sites or cells. Therefore, although an odour may be perceived in a mixture, it may not be identifiable, or lowering of the original intensity and information about one or both odorants can be lost (Derby et al. 1991; Laing 1992).

1.4.2.2 Temporal filtering

The concept of temporal filtering in odour mixture perception was proposed by Laing (1987). Thus some odours differ greatly in the latency time to stimulate receptor cells, with differences in the order of hundreds of milliseconds recorded. Consequently, if two odours with different stimulating latency times are present, information about one odorant is likely to reach the brain faster with odour memory and identification before

another. In theory the "faster" odour will occupy more olfactory resource and reduce the chance of stimulation by a slower odorant, or may simply act as an antagonist and block entry to receptor sites by the second odour. 'Fast' odorants, therefore, have a distinct advantage over slow odorants and are more likely to suppress the perceived intensity of a slower odorant and change the characteristic response pattern of the latter odorant beyond identification (Laing 1992).

1.4.3 Psychological perspective

All living organisms can detect and identify a wide range of chemical substances in their environment. A unique odour can trigger distinct memories from our life experience or from emotional moments, positive or negative (Davis et al. 2007). For example, illness caused by rotten meat can leave a memory that stays with us for years, and prevent us from ingesting it again, whereas a good whisky or a fresh strawberry activates a whole array of odorant receptors, helping us to detect the qualities of that product that we regard as positive. Sensory perception, expectations, and eating habits vary from individual to individual, depending on their age, sex, culture, and previous experiences. Hence, the same food may be perceived as tasting differently by two individuals or by a single individual at different times (Axel and Buck 2004; McClements 2004).

1.5 Whisky analysis

1.5.1 Analytical methods

Whisky contains many hundreds of congeners, including alcohols, acids, esters, carbonyl compounds, phenols, hydrocarbons, and trace amounts of nitrogen and sulphur containing compounds. These congeners are analysed at the mg/L, µg/L and even ng/L levels (Swan 1981). All of these aroma congeners are natural constituents of the production process, with some clearly contribute sensory character and some others do not. However, together all the congeners help to make each whisky unique.

1.5.1.1 Phenol analysis

Phenolic congeners are usually determined by direct-injection reversed phase gradient

HPLC with fluorescence detection (Aylott 2003). Alternatively, a better separation, sensitivity and selectivity may be achieved by using capillary column gas chromatography but longer sample running time may be required (Lehtonen 1983a).

In addition, the phenol measurements can also be carried out using solid phase micro-extraction (SPME) combined with GC/MS. SPME has several advantages for volatiles analysis in complex matrices such as food and beverages. One of the most important properties of SPME is directly trapping the aroma congeners from the sample headspace, as the working mechanism is analogous to the human nose detecting aroma (Lehtonen 1983b).

1.5.1.2 Distillate congeners analysis

The volatile congeners in whisky can be conveniently subdivided into major and trace congeners. Typically, congener analyses are usually measured by gas chromatography (AOAC 2000; Aylott 2003), which is applied widely in whisky production and research. For example, it may be used in the competitor product analysis to determine the percentage of malt whisky used in a blended Scotch whisky and in consumer protection activity to confirm brand authenticity or used to monitor the efficiency of rectification in the continuous stills used to distil Scotch grain whisky (Aylott et al. 1994; Aylott 2003; MacKenzie and Aylott 2004).

1.5.1.3 Maturation-derived compounds

Cask-derived compounds, namely lignin degradation products and polyphenolics, can determined by HPLC (Lehtonen 1983a; Lehtonen 1983b). Direct-injection reversed-phase gradient elution HPLC with ultraviolet and/or fluorescence detection are particularly appropriate for this analysis (Aylott et al. 1994; Axel and Buck 2004).

1.5.1.4 Gas Chromatography – Olfactometry (GC-O)

Gas chromatography is a powerful separation method in analytical chemistry. Nowadays it is common to apply GC/MS (gas chromatography-mass spectrometry), to aid identification of the volatile component that is detected by simultaneous

olfactometry (Cuyper and Bulte 2001; Blank 2002). However, GC/MS is an indirect method of measurement and does not attempt to analyse the individual odour-active compounds. Based on previous research knowledge of aroma chemistry only a small fraction of volatiles present in food are odour-active (Belitz et al. 2004). In order to identify the odour-important compounds we should ideally use a bioassay to represent the pattern of odorants in terms of their aroma-activity instead of concentration such that the data reflects the odour potential of the chemical and eliminates the odourless compounds from the analysis.

GC-O Principle

GC-O is carried out by installing at the end of a chromatographic column a split which allows the sample to be split 1:5, between a detector, such as an FID detector and a odour port (Jirovetz et al. 2002). The peak/odours correlation can then be performed by experienced assessors for best results. The human nose is often more sensitive than any physical detector, and GC-O exhibits powerful capabilities that can be applied to aromas and any odoriferous products (Jirovetz et al. 2002; Van Ruth and Roozen 2004) During GC-O an extract or distilled sample from food matrices is injected into a GC that has been modified with an GC/MS-olfactometer at the detector end. A sniffer or human detector will sit at the olfactometer outlet and record what they smell as it is detected in a humidified air stream (Friedrich and Acree 2000).

GC-O analysis has some drawbacks, many of which are related directly to the use of a human as a detector. GC-O is time-intensive and typically only 1-2 panellists are used (Abbott et al. 1993) who must be pre-screened for sensitivity and specific anosmia. Also, it is often difficult for a sniffer to detect the end of an odour region. It also been shown that the olfactory sensitivity of an individual changes throughout the day as well as over longer periods of time (Friedrich and Acree 2000). However, panellists can be trained to consistently identify smells within short periods of standardized time (Friedrich and Acree 2000).

1.5.1.5 Identification of potent odorants by GC-O Aroma Extract Dilution Analysis

The most aroma-active compounds usually do not correspond to the major volatile

components in the food, and indeed some of these aroma-active compounds are extremely potent and exist is such small amount that they cannot be detected by typical GC detectors (Blank 2002). This can be explained by the low odour threshold of these compounds. Identification of such minor components is a challenging task. An example is the identification of 1-p-menthene-8-thiol as the principal aroma of grapefruit juice (Mussinan and Keelan 1994), its threshold is the lowest reported for a naturally occurring compound: 2×10^{-8} mg/l water (Blank 2002).

Detection of odorous regions in a gas chromatogram is the first useful information that can be obtained from a single GC-O run. In the first GC-O run, all volatiles are detected whose concentrations in the GC effluent are higher than their odour thresholds (Blank 2002). The corresponding volatiles are then characterized by their aroma quality and intensity as well as by their chromatographic properties (e.g. retention index (RI)). Once the aroma-active regions have been selected by a dilution analysis, the often time-consuming identification experiments can be focused on the most potent odorants. Verification by GC/MS is possible by tuning the detection technique, e.g. searching for typical fragments of the target molecule, and in a well-defined region of the gas chromatogram, recording in SIM (selected ion monitoring) mode (Blank 2002).

It is very difficult to judge the sensory relevance of volatiles from a single GC-O run. Several techniques have been developed to objectify GC-O data and to estimate the sensory contribution of single aroma components. Dilution techniques and time-intensity measurements are the two main GC-O methods (Drake et al. 2007). Dilution methods are based on successive dilutions of an aroma extract and re-evaluation until no odour is perceived at the sniffing port of the chromatograph. Intensity methods rely on the assessor recording the odour intensity as a function of time for a single assessment of an aroma extract. Various input devices have been used for such time-intensity methods, including the application of a variable resistor that the assessor attempts to move in line with their perceived sensory intensities (Blank 2002).

General approaches to the identification of “important” or high-impact odorants are based on odour activity values (OAV). GC-O facilitates the process of determining OAVs, and elucidation of the most odour-active compounds are generally achieved

using a well-known dilution analysis technique, GC-O aroma extract dilution analysis (AEDA) (Ryan et al. 2008). In AEDA, the assessor indicates whether or not an aroma can be perceived and notes the sensory descriptor. The results are expressed as the aroma dilution (FD) factor that corresponds to the maximum dilution value detected. The FD factor is a relative measure and represents the odour threshold of the compound at a given concentration. AEDA has been proposed as a screening method for potent odorants as the results are not corrected for losses during isolation(Blank 1996).

1.5.2 Sensory analysis

In common with other foods and drinks, improved analytical measurements have not provided substantial help in understanding of the complex aroma of whisky (Conner et al. 2001). One of the main factors is that, so far, aroma scientists still cannot predict the aroma perception outcome of a dynamic interaction between individuals and aroma components. Obviously, only relying on chemical analysis is not enough, so sensory-based methods are needed in conjunction with analytical chemistry to provide a bioassay for possible sensory impact.

Over the past 50 years, sensory analysis in the whisky industry has made considerable progress. Sensory analysis has grown into a powerful tool for whisky aroma assessment, and approaches such as scaling, descriptive analysis and threshold determination make it possible to answer a wide variety of questions (Jounela-Eriksson 1983). One aspect in common to all sensory test methods is that they use humans as the measuring instrument (Lawless and Heymann 1998b). Sensory analysis requires an 'instrument', which is always one or more people. The human instruments can be usefully divided into three types: consumers, research panels and small expert groups (Dürr 1989).

1.5.2.1 Quantitative Descriptive Analysis

Quantitative descriptive analysis (QDA) is an important sensory analysis technique as it allows communication of data on aroma characters in a specific product, and has been used to provide a measure of the relative intensity of a range of common whisky

sensory attributes. QDA was developed during the 1970s to improve the perceived problems associated with aroma profile analysis (Lawless and Heymann 1998a). Most importantly for QDA systems, a vocabulary must be developed, which must be sufficiently large to include all aroma notes likely to be encountered, but sufficiently small to ensure ease of use. Each word in the descriptive vocabulary must be precisely defined (Harper et al. 1968). A characteristic description aroma wheel for expressing the aroma of Scotch whisky has been developed (The Scotch whisky Research Institute's Aroma Wheel; as shown Figure 1.8), which illustrates the complexity of Scotch whisky aroma and the spectrum of attributes that can contribute to overall character (Lee et al. 2001b; Aylott 2003; Jack 2012).

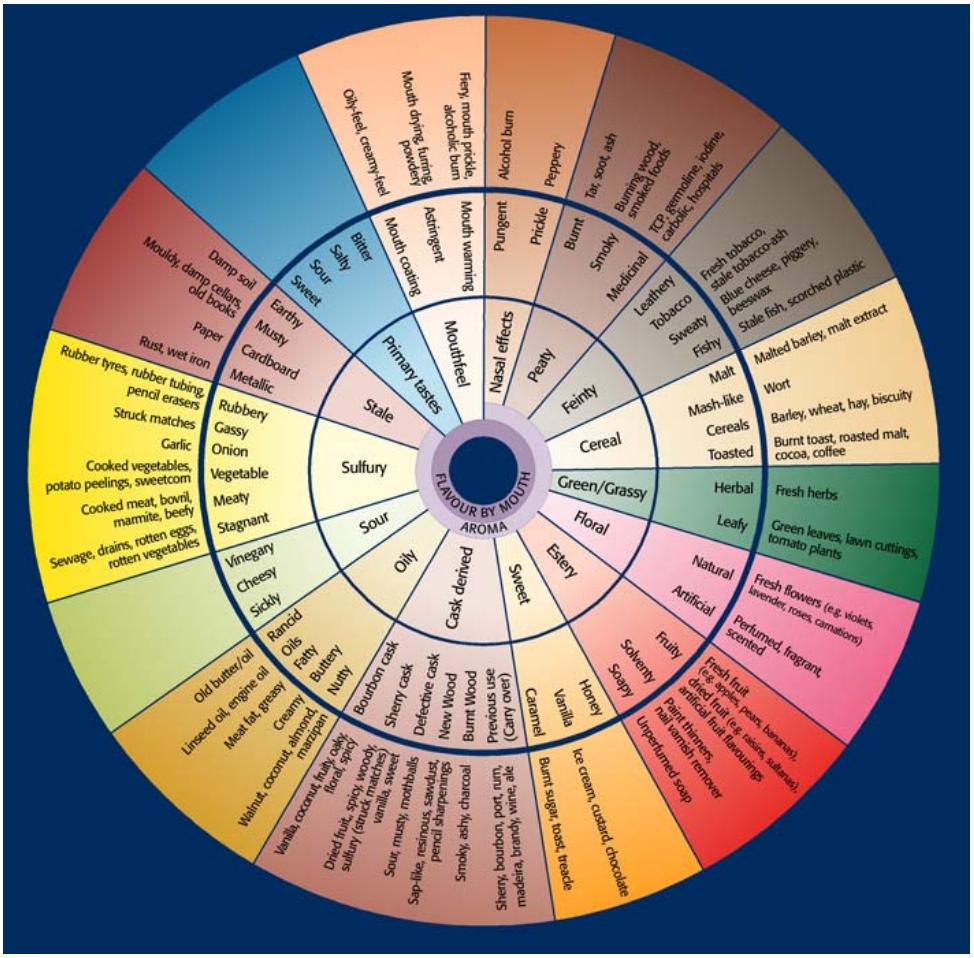


Figure 1.8 – The Scotch whisky Research Institute's Aroma wheel after (Lee et al. 2001).

1.5.2.2 Thresholds determinations

Thresholds are commonly used to measure the intensity of perceived sensory response,

they have been employed to estimate the relative aroma potential of components by assuming that the lower the detectable concentration of a compound, the more likely it is that it contributes to the aroma of the product (Jounela-Eriksson 1983). Thresholds are the limits of sensory capacities. It is convenient to distinguish between the absolute threshold, the recognition threshold, the difference threshold, and the terminal threshold (Thornton 1987).

1. **The absolute threshold** (detection threshold or absolute limit) is the smallest value of a stimulus that an observer can detect, the odorant concentration which has a probability of 0.5 (50% assessors) of being detected under the conditions of the test. In other words, the absolute threshold is the lowest stimulus capable of producing a sensation e.g. the dimmest light, the softest sound, the lightest weight, the weakest aroma.

2. **The recognition threshold** is the level of a stimulus at which the specific stimulus can be recognized and identified, the odour concentration which has a probability of 0.5 (50% assessors) of being recognized under the conditions of the test. The recognition threshold is usually higher than the absolute threshold. In food research, the recognition threshold for a given aroma in a food would be a more useful thing to know than the absolute threshold.

3. **The terminal threshold:** The terminal threshold or region is the magnitude of a stimulus above which there is no increase in the perceived intensity of the appropriate quality for that stimulus. In other words, the sensory response has reached some saturation level, beyond which no further stimulation is possible due to maximal response of receptors or nerves to some physical process limiting access of the stimulus to receptors.

4. **The difference threshold** or just-noticeable difference (JND) is the smallest change in a stimulus which the observer can detect. The difference threshold is determined by changing the stimulus by varying amounts to see whether a subject can perceive any difference in the stimulus.

Threshold is defined as a concentration at which a stimulus is correctly detected by 50% (probability of 0.5) of assessors (Lawless and Heymann 1998b). This is because thresholds are not fixed points, but values on a stimulus continuum. If the conceptual threshold were a fixed physical quantity, we would have a situation where below this range the stimulus is nearly never perceived, and above this range it is nearly always perceived. Thresholds are often considered as fundamental concepts in food aroma research by many aroma chemists and psychologists. Thresholds have the units of concentration, e.g. mg/L or moles per litre, of the compound in a specified base product (Meilgaard 1975; Lawless and Heymann 1998a). The threshold value is greatly influenced by many factors such as measurement procedures, test methods, statistical interpretation, sensory and non-sensory factors (Meilgaard 1975; Lee et al. 1999a), individual differences in thresholds and test medium (e.g. water or actual foods and beverages).

1.5.2.3 Odour unit

An odour unit is a commonly used sensory unit indicates how a single compound contributes to the overall aroma character and is calculated as the ratio of the concentration of a compound to its threshold value. Meilgaard (1975) was the first researcher to study the sensory contribution of single volatiles to alcoholic beverages by determining the threshold of aroma compounds and the corresponding Odour units. And the odour unit calculation using Equation 1(Lawless and Heymann 1998a).

$$\boldsymbol{Odour\ Unit\ (O) = \frac{Concentration\ (C)}{Threshold\ (FIC)}} \qquad \textbf{Eq. 1}$$

Theoretically, compounds with Odour Units < 1 are not expected to contribute to the overall aroma. In practice, it is not this simple, rather the overall aroma character of the whisky matrix is the sum of the contributions of its individual compounds (Meilgaard 1975; Fritsch and Schieberle 2005). As previously reported, many interactions can occur between aroma compounds that could have impacts on aroma (Poisson and Schieberle 2008)

1.5.3 Statistical analysis

Efforts to correlate analytical and sensory data are commonly made by using

statistical analysis to identify the components or parameters which relate to the variation of sensory characteristics (Jounela-Eriksson 1983).

1.5.3.1 Principal component analysis

Principal Component Analysis (PCA) is used to simplify extensive data sets into an easily visualized format (Jolliffe 1986). The idea of this technique is the creation of fewer, new variables that explain as much of the information in the data as possible. Formally the methodology attempts to reduce the dimensions of data by creating new, uncorrelated axes or principal components from the initial variables in the data set. These new variables are linear combinations of the original variables. The first principal component is selected to explain the largest amount of the variance in the data set. The second component both explains the largest amount of remaining variance in the data set and is orthogonal (ie independent) to the first principal component. The third component follows and so on as described before. Therefore, every principal component describes different information from the other. The number of principal components equal the number of the original variables but they are weighted differently (Jolliffe 1986). Depending on the situation, it is often possible to use fewer PCs to give an indication of the interrelationships within a data set, a phenomenon known as reduced dimensionality. The greater degree of intercorrelation between the initial variables, that greater the opportunity for reduced dimensionality.

1.5.3.2 Analysis of Variance

Analysis of Variance (ANOVA) allows comparisons to be made between any number of sample means. Every statistical test is based on a hypothesis that presumes that there is no difference between the sample means. This is called the 'Null Hypothesis' and has p-value bigger than a set value, typically 0.05 (the value for 95% confidence). An alternative hypothesis states that there is a difference between samples and, in this case, a p-value smaller than 0.05. Depending on the calculated probability, only one of two hypotheses is accepted (Lea et al. 1997; Fowler 1998).

1.5.3.3 Regression analysis

Regression analysis is widely used in prediction and forecasting based on the

information provided while focusing on the relationship between one dependent variable and one or more independent variables. The relationship of two parameters is often illustrated by the use of a scatter plot. When a scatter plot is presented, it is usually helpful to draw a line of best fit (Fowler 1998) through the points so that their average relationship can be described. A problem arises as to how to fit the line to the scatter plot. In some cases, the line can be fitted by eye, but in other cases a mathematical approach is used to give the 'regression line'. Based on geometry, the equation $y = a + bx$ describes a straight line. Regression analysis solves for the values '*a*' and '*b*' in the equation from a set of data. It is then feasible to fit a line in a scatter plot and calculate the value of one variable from the other. The parameters '*a*' and '*b*' are the regression coefficients. The R^2 (R squared) value should always be checked to see how well the regression equation models the observed data. It can have values between 0 and 1, with 1 indicating a perfect fit and 0 indicating random scatter. The larger the R^2 value, the better the proposed model describes the relationship between the dependent and independent variables (Fowler 1998).

1.6 Aims and Objectives

Most blenders believe that aroma interactions occur during whisky blending, with some aromas being thought to be suppressed with other aromas being enhanced in the blend. Nowadays, in the whisky industry, the blending process has developed as an art rather than a science and relies heavily upon the experience and judgment of expert individuals or Master blenders. Whisky aroma interactions during blending practice remain largely unexplored and the establishment of criteria for assessing blending aroma interactions is a difficult task without any quantitative controls.

The objective of the present research was to establish a means of prediction and control of aroma in whisky blending. The primary aim was to assess the impact of woody, malt and grain whisky character (components commonly used in blended whisky) on the perception of peaty character. Future detailed aims of this research are outlined below:

1. To determine whether or not the perception of peaty aromas are influenced (synergistic or antagonistic interactions) by the other constituent whiskies in the blend.

2. To determine if any observed peaty aroma interaction are due to physiochemical or physiological effects.

3. To develop a method to quantify and measure the peaty aroma masking effect in different whisky matrices.

4. To establish models to predict the intensity of peaty character in the whisky blend.

5. To verify the prediction model based on laboratory made blends.

CHAPTER 2. MATERIALS AND METHODS

2.1 Samples and materials

2.1.1 Chemicals and solvents

2.1.1.1 Phenolic compounds

Phenolic compounds were purchased from Sigma-Aldrich Company Ltd (Gillingham, Dorset, UK, SP8 4XT). The relevant information of these phenolic compounds is shown in Table 2.1.

Table 2.1 – Purity, aroma character and threshold values of phenolic compounds (Leffingwell & Associates 2014).

Phenolic Compounds			
Compounds	**Aroma character**	**Threshold[1] (mg/l)**	**Purity (%)**
(P1) guaiacol	smoky, medicinal, woody, bacon	0.04	99.0
(P2) 4-methylguaiacol	spicy, phenolic, sweet, clove-like	0.95	97.6
(P3) *o*-cresol	phenolic	0.61	99.0
(P4) phenol	phenolic, medicinal, antiseptic	19.2	99.5
(P5) 4-ethylguaiacol	spicy, smoke-like, sweet, vanilla	0.11	98.2
(P6) *p*-cresol	phenolic, aromatic, slightly spicy	0.05	98.6
(P7) *m*-cresol	phenolic	0.58	99.0
(P8) 4-ethylphenol	phenolic, aromatic slightly spicy	0.47	99.0
2,3,5-trimethylphenol*	phenolic	-----	99.0

*2,3,5-trimethylphenol was used as internal standard in the study, because it not naturally produced in whiskies production

.

[1] Threshold values were obtained from SWRI thresholds database.

2.1.1.2 Major volatile congeners

Major congeners were purchased from Greyhound Chromatography and Allied Chemicals Company Ltd. (Preston, Merseyside, U.K., CH43 4XF). The aroma character, the threshold values and the % purity of these major volatile congeners are shown in Table 2.2.

Table 2.2 – Purity, aroma descriptions and threshold values of major volatile congeners.

Higher Alcohol and Major Aroma Volatiles			
Compounds	**Aroma character[2]**	**Threshold[3] (mg/l)**	**Purity (%)**
(D1) acetaldehyde	pungent, ethereal, fresh on dilution	12	99.5
(D2) ethyl acetate	ethereal, fruity	74	99.5
(D3) acetal	strong, tart, fruity	4	99.5
(D4) methanol	alcoholic, fruity	---	99.5
(D5) n-propanol	alcoholic, sweet	>3000	99.5
(D6) iso-butanol	ethereal, fermented, yeasty	700	99.5
(D7) iso-amyl acetate	estery, fruity, banana, pear, sweet	1.5	99.5
(D8) n-butanol	medicine, fruit, wine	500	99.5
(D9) 2-methyl-1-butanol	malt, whisky-like	250	99.5
(D10) 3-methyl-1-butanol	whisky, malt, burnt	300	99.5
n-pentanol	Used as internal standard	---	99.5

[2] Aroma character was taken from the Basic 98 database of aroma (raw) materials.
[3] Threshold values were obtained from SWRI thresholds database.

2.1.1.3 Trace volatile congeners

Trace congeners were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, U.K., SP8 4XT). The aroma character, the threshold values and the % purity of these trace volatile congeners are shown in Table 2.3.

Table 2.3 – Purity, aroma descriptions and threshold values of trace volatile congeners.

Esters and Acids			
Compounds	**Aroma character[4]**	**Threshold[5] (mg/l)**	**Purity (%)**
(D11) ethyl hexanoate	powerful, fruity, wine-like	0.2	99.5
(D12) ethyl octanoate	floral, banana, pineapple	0.4	99.5
(D13) ethyl decanoate	brandy, oily, fruity, grape	2	99.5
(D14) ethyl dodecanoate	fruity, oily-fatty	2	99.5
(D15) ethyl tetradecanoate	sweet, waxy, creamy	180	99.5
(D16) ethyl hexadecanoate	soft, waxy, fatty	3000	99.5
(D17) ethyl 9-hexadecenoate	strongly fatty	---	99.5
(D18) 2-phenethyl acetate	rose, honey, tobacco	---	99.5
(D19) 2-phenethyl alcohol	rose, honey	---	99.5

[4] Aroma character was taken from the Bacis 98 database of aroma (raw) materials.
[5] Threshold values were obtained from SWRI thresholds database.

2.1.1.4 Maturation derived compounds

Maturation derived compounds were purchased from Sigma-Aldrich Company Ltd. (Gillingham, Dorset, U.K., SP8 4XT). The aroma character, the threshold values and the % purity of these maturation derived compounds are shown in Table 2.4.

Table 2.4 – Purity, aroma descriptions and threshold values of maturation derived compounds.

Maturation Derived Compounds			
Compounds	**Aroma character[6]**	**Threshold[7] (mg/l)**	**Purity (%)**
(W1) gallic acid	-----	odourless	98
(W2) ellagic acid	-----	odourless	95
(W3) coniferaldehyde	-----	odourless	98
(W4) vanillin	sweet, vanilla	0.17	98
(W5) vanillic acid	sweet aromatic, like vanilla	100	99.7
(W6) sinapaldehyde	-----	odourless	98
(W7) syringaldehyde	grape; woody; smoky	50000	99
(W8) syringic acid	-----	odourless	95
(W9) scopoletin	-----	odourless	99
(W10) 5-hydromethylfurfural	mild, soft, ethereal, caramellic	450	99

2.1.1.5 Solvents

Ethanol was purchased from McQuilkin & Co. (College Milton North, East Kilbride, U.K., G74 5HD). Ultra High Quality (UHQ) water was produced using an ELGA LabWater Purelab UHQ 11 purification system (ELGA LabWater Global Operations, U.K., HP14 3BY).

[6] Aroma character was taken from the Basic 98 database of aroma (raw) materials.
[7] Threshold values were obtained from SWRI thresholds database.

2.1.2 Whisky samples

2.1.2.1 Industry supplied whisky samples

The following whisky samples were used as blending matrices (Diageo Ltd., London, U.K.) – the standard and woody grains, and unpeated malt are blended with the peated malt Caol Ila for aroma interaction measurement experiments (Table 2.5).

Table 2.5 – Whiskies used in sensory and analytical experiments.

Industry supplied whisky samples					
Whisky	**Type**	**Location**	**Alcohol Strength (v/v)**	**Age (years)**	**Cask type**
Caol Ila* (*peated malt*)	Peated malt	Islay	40%	3	Refill
Vatted malt*# (*unpeated malt*)	Unpeated malt	Speyside	40%	3	Refill
Glendullan	Unpeated malt	Speyside	40%	3	Refill
Linkwood	Unpeated malt	Speyside	40%	3	Refill
Clynelish	Unpeated malt	Highlands	40%	3	Refill
Cardhu	Unpeated malt	Speyside	40%	3	Refill
Blair Athol	Unpeated malt	Highlands	40%	3	Refill
Knockando	Unpeated malt	Speyside	40%	3	Refill
Dailuaine	Unpeated malt	Speyside	40%	3	Refill
Benrinnes	Unpeated malt	Speyside	40%	3	Refill
Vatted grain*# (*Standard Grain*)	Standard grain	Lowlands	40%	3	Refill
Cameron Bridge	Standard grain	Lowlands	40%	3	Refill
Port Dundas	Standard grain	Lowlands	40%	3	Refill
Girvan	Standard grain	Lowlands	40%	3	Refill
Invergordon	Standard grain	Highland	40%	3	Refill
Vatted Woody grain* (*Woody Grain*)	Woody grain	Lowlands	40%	3	Ex-Bourbon
Cameron Bridge (2)	Woody grain	Lowlands	40%	2	Ex-Bourbon
Cameron Bridge (4)	Woody grain	Lowlands	40%	4	Ex-Bourbon
Cameron Bridge (7)	Woody grain	Lowlands	40%	7	Ex-Bourbon
Cameron Bridge (9)	Woody grain	Lowlands	40%	9	Ex-Bourbon
Cameron Bridge (12)	Woody grain	Lowlands	40%	12	Ex-Bourbon

* Four basic type whiskies

vatted means several smaller category spirits blended together

2.1.2.2 Commercial whisky samples

The following commercial peated whiskies were used in both sensory and analytical measurements. All commercial whisky samples (Table 2.6) were purchased from Speciality Drinks Ltd, London, U.K.

Table 2.6 – Commercial whiskies used in sensory and analytical experiments.

Commercial whisky samples					
Whisky	**Type**	**Location**	**Alcohol Strength (v/v)**	**Age (years)**	**Cask type**
Laphroaig	Peated malt	Islay	46%	7	Multi-cask*
Bunnahabhain	Peated malt	Islay	46%	9	Multi-cask*
Ardbeg	Peated malt	Islay	45.8%	10	Multi-cask*
Lagavulin	Peated malt	Islay	46%	16	Multi-cask*
Highland Park	Peated malt	Island	40%	12	Multi-cask*
Talisker	Peated malt	Island	43%	18	Multi-cask*

**Whisky has been maturated in more than one cask*

2.2 Analytical evaluation

2.2.1 Phenol analysis by headspace solid phase micro-extraction gas chromatography mass spectrometry

Headspace Solid Phase Micro Extraction in conjunction with GC/MS (HS-SPME-GC-MS) was used to determine the difference of headspace phenol level between different types of whiskies. A typical phenol headspace analysis chromatogram obtained from a peated malt (Caol Ila) is shown in Figure 2.1.

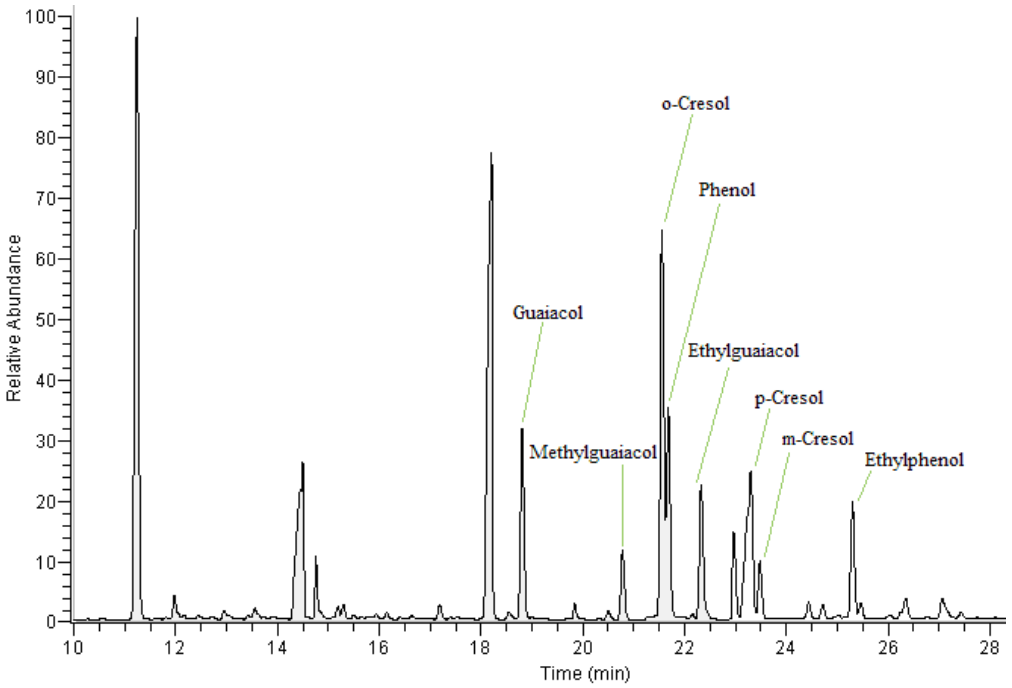


Figure 2.1 – Chromatogram produced from HS-SPME-GC/MS analysis of Scotch peated malt, screenshot from Caol Ila analysis.

Standard preparation

Eight individual stock standard solutions were prepared in 70% (v/v) ethanol/UHQ water solution with a range of phenols, namely: (P1) guaiacol, (P2) 4-methylguaiacol, (P3) *o*-cresol, (P4) phenol, (P5) 4-ethylguaiacol, (P6) *p*-cresol, (P7) *m*-cresol and (P8) 4-ethylphenol. These eight individual stock standard solutions were then mixed in 70% (v/v) ethanol as a mixed phenol standard, with each phenol concentration at approximately 20-30 mg/l. Six calibration standards were prepared by diluting the mixed phenol standard to create a six point calibration curve.

In sensory analysis, all of the samples were diluted down to around 20% (v/v) ethanol as this was the usual alcohol strength for whisky sensory tests (Jack, 2012). Therefore, to maintain the same analytical conditions between the sensory analysis and headspace SPME-GC/MS measurements, 2 ml of the calibration standards were then diluted with 4 ml UHQ water into 10 ml sample vials (to give total of 6 ml). Analysis used an internal standard, 2,3,5-trimethylphenol, which was prepared in 70% (v/v) ethanol/UHQ water solution of 100 mg/l, and 50 µl of internal standard was added to each calibration standard.

Whisky samples preparation

Three ml of whisky samples (40% (v/v)), were added to 10 ml standard vials and were diluted to 20% (v/v) with 3 ml UHQ water. 50 µl of internal standard solution of approximately 100 mg/l of 2,3,5-trimethylphenol was added to all samples.

Analytical instrumentation

Analyses were carried out by GC/MS (Thermo Fisher Scientific Inc., Waltham, MA 02454, USA). The column used was a 60 m x 0.32 mm DB-Wax capillary column with a film thickness of 0.5 µm. The carrier gas was He at a flow-rate of 1.4 ml min^{-1}. The initial oven temperature was 40°C, held for 1 minute, increasing to 250°C at 5°C min^{-1} with a final hold time of 11 min. The injector temperature was maintained at 240°C. The transfer line temperature was maintained at 250°C. The mass spectrometer was operated in the electron impact (EI) mode and ions from 35 to 400 amu were scanned at a rate of 2 scans s^{-1}.

Sampling condition

The pre-incubation time for the samples was 5 min at 30°C. Extraction time was 15 min. The SPME fibre used was an 85 µm of polyacrylate (PA) (Supelco UK, Gillingham, Dorset, UK, SP8 4XT) as this fibre has previously been found to be useful for the analysis of phenols (Buchholz and Pawliszyn 1993).

2.2.2 Distillate congeners analysis by gas chromatography

The concentration of distillate aroma congeners in the different types of whiskies was measured by gas chromatography (GC).

2.2.2.1 Major distillate congeners analysis by gas chromatography

A typical major congener analysis by GC chromatogram obtained from unpeated malt is shown in Figure 2.2.

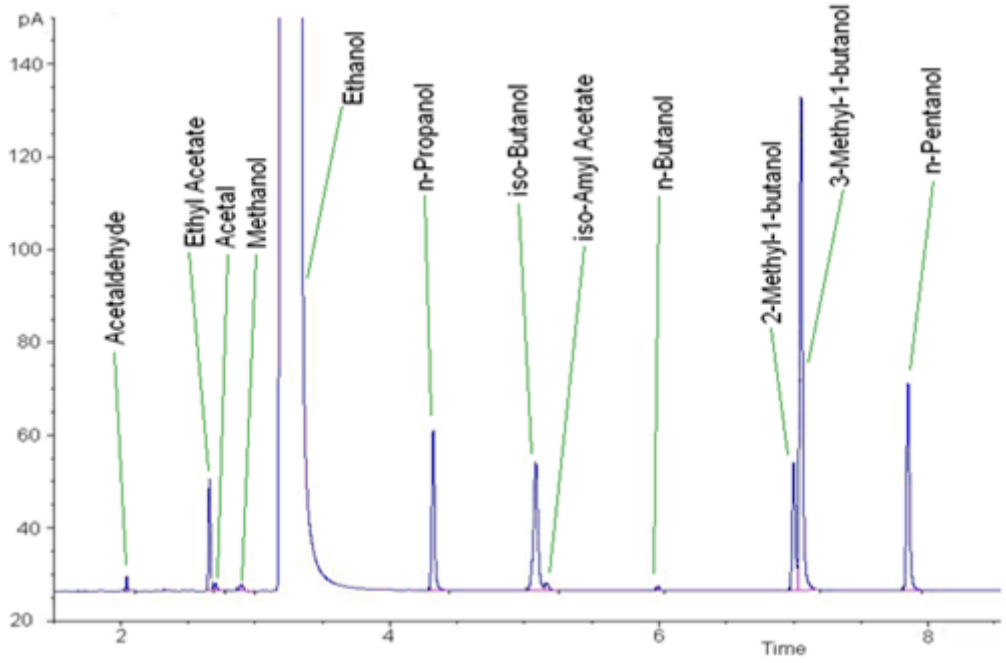


Figure 2.2 – Chromatogram produced from major congener GC analysis, screenshot from unpeated malt analysis.

Standard preparation

A total of nine stock standard solutions were prepared in 40% (v/v) ethanol solution with a range of major volatile congeners namely (D1) acetaldehyde, (D2) ethyl acetate, (D3) acetal, (D4) methanol, (D5) n-propanol, (D6) iso-butanol, (D8) n-butanol, (D9) 2-methyl-1-butanol and (D10) 3-methyl-1-butanol (Kahn 1969; Kahn and Blessinger 1972; Kahn 1979; Ebeler 2004). In this study, the major volatile congeners were analysed by direct injection gas chromatographic separation (Kelly et al. 1999). These individual stock standards were then mixed in 40% (v/v) ethanol, and eight mixed calibration standards were finally prepared covering the range 5 to 1250 µg/ml for each analyse. The internal standard, n-pentanol, was prepared with 40% (v/v) ethanol solution to approximately 5000 µg/ml internal standard solution, 50 µl of internal standard was added to each mixed calibration standard.

Sample preparation

For major volatile congener analysis, all the samples were analysed at 40% (v/v) ethanol. Therefore, 1 ml of whisky samples 40% (v/v) were pipetted into 2 ml crimp top GC vials, then 50 µl of internal standard solution (5000 µg/ml) was added.

Analytical instrumentation

Analyses were performed by gas chromatography (Hewlett Packard 6890 Series Gas Chromatograph fitted with an autosampler). The column used was a 50 m x 0.25 mm Chrompack CP WAX CB 57 column, internal diameter film thickness of 0.2 µm. The carrier gas was hydrogen with constant pressure 6 psi. Oven temperature was 35°C initially, and was raised to 190°C at 7°C/min. The temperature was held at 210°C for 3 minutes post run. 0.5 µl sample was injected with split (1:15) and injector temperature was maintained at 200°C. The flame ionisation detector was operated at temperature of 250°C. HP Chemstation Software was used for computer control, data acquisition and reprocessing of data. Integration parameters were stored in the Chemstation software and were adjusted according to the instrument performance.

2.2.2.2 Trace distillate congener analysis by gas chromatography

A typical trace congener analysis by GC chromatogram obtained from unpeated malt is shown in Figure 2.3.

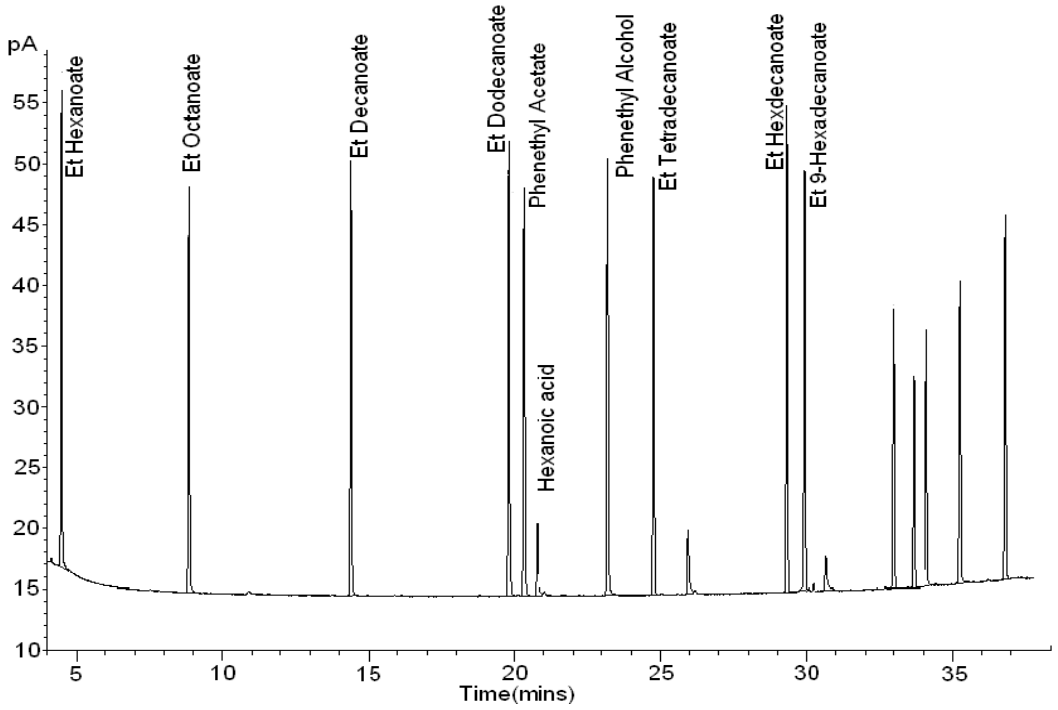


Figure 2.3 – Chromatogram produced from trace congener GC analysis, screenshot from unpeated malt analysis.

Standards preparation

The stock standard solutions were prepared with a range of esters and free fatty acids – (D11) ethyl hexanoate, (D12) ethyl octanoate, (D13) ethyl decanoate, (D14) ethyl dodecanoate, (D15) ethyl tetradecanoate, (D16) ethyl hexadecanoate, (D17) ethyl 9-hexadecenoate, (D18) 2-phenethyl acetate and (D19) 2-phenethyl alcohol. In this study, the esters were also analysed by chromatographic separation and methyl octadecanoate was used for internal standards.

All the analytes were prepared in 70% (v/v) ethanol to create the individual stock standard solutions at a concentration about 1000 μg/ml, with the exception of (D16) ethyl hexadecanoate and ethyl octadecanoate which were made up in 100% (v/v) ethanol. Then the individual stock standard solutions were diluted in 70% (v/v) ethanol to create 8 mixed calibration standards over the range 1 - 50 μg/ml for each analyte. The internal standard, methyl octadecanoate, was prepared in methanol to approximately 100 μg/ml internal standard solution; 100 μl of internal standard was added to each mixed calibration standard.

Sample preparation

Since all samples were analysed at strength as near to 70% (v/v) ethanol/water as possible, 500 μl of whisky samples (40% (v/v) ethanol were pipetted into 2 ml crimp top autosampler vials. Then 500 μl of ethanol and 100 μl of methyl octadecanoate internal standard solution were added.

Analytical instrumentation

Analyses were performed by gas chromatography (Hewlett Packard 6890 Series Gas Chromatograph fitted with an autosampler). The column used was a 30 m x 0.32 mm Stabilwax-DA column, i.e. film thickness 0.5μm. The carrier gas was hydrogen with constant pressure 6 psi. The initial oven temperature was 50°C, held for 1 minute, increasing to 160°C at 4°C min^{-1}, then increasing to 190°C at 3.5°C min^{-1} with a final increase to 240 at 40°C min^{-1}. Samples of 0.5 μl were injected with splitless injection and injector temperature maintained at 240°C. The flame ionisation detector was operated at temperature 250°C. HP Chemstation Software was used for computer control, data acquisition and reprocessing of data. Integration parameters were stored in the Chemstation software and adjusted according to the instrument performance.

2.2.3 Maturation derived compounds analysis by HPLC

HPLC with direct injection was used to analyse the ten quantitatively important maturation derived compounds to determine the maturation related compound levels in different types of whisky. A typical maturation derived compound analysis chromatogram obtained from woody grain is shown in Figure 2.4.

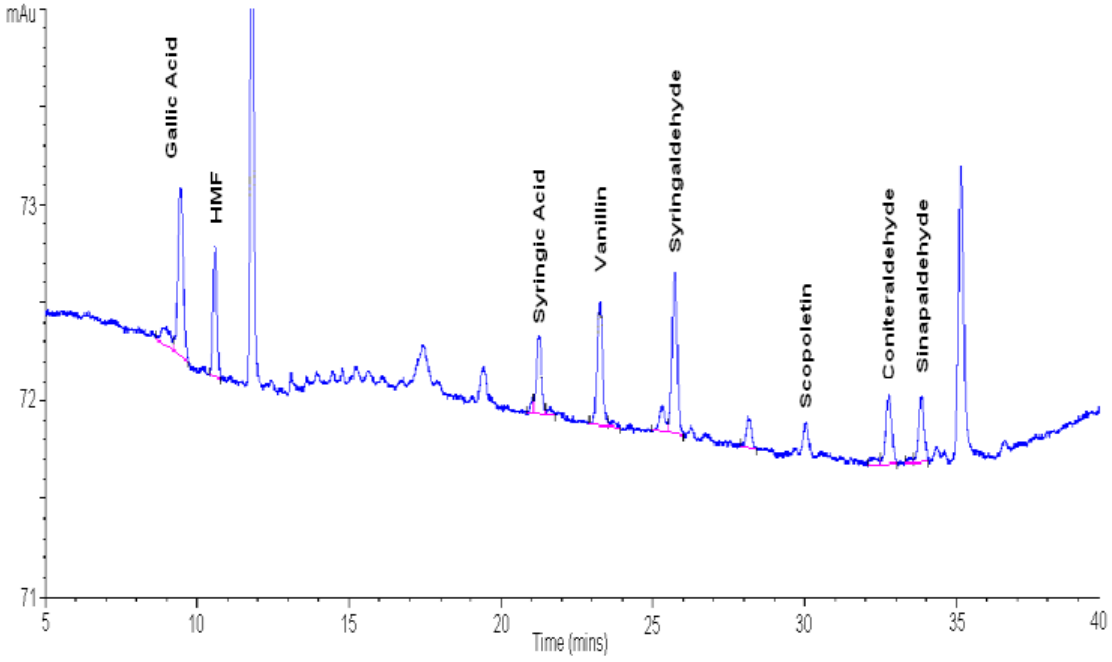


Figure 2.4 – Typical maturation derived compounds HPLC chromatogram produced from screenshot of woody grain analysis.

Standard preparation

Stock standard solutions were prepared for a range of maturation derived compounds including: (W1) gallic acid, (W2) ellagic acid, (W3) coniferaldehyde, (W4) vanillin, (W5) vanillic acid, (W6) sinapaldehyde, (W7) syringaldehyde, (W8) syringic acid, (W9) scopoletin and (W10) 5-HMF. Approximately 60 mg of each analyte was weighted accurately and dissolved in a 100 ml volumetric flask with 1% (v/v) orthophosphoric acid in 80% (v/v) ethanol diluent to create the individual stock standard solutions. For ellagic acid approximately 50 mg was dissolved to a final volume of 500 ml in 1% (v/v) orthophosphoric acid in methanol. Then the individual stock standard solution was diluted with 1% (v/v) orthophosphoric acid in 80% (v/v) ethanol/water to create six mixed calibration standards over the range from 0.3 to 30 µg/ml for each analyte. Analyte quantification was by an external standard method for each compound over a range of mixed calibration standards.

Sample preparation

All samples for analysis were manually shaken to ensure homogeneity and were filtered directly into a vial through a syringe filter prior to HPLC analysis.

Analytical instrumentation

Analysis was carried out on Hewlett Packard Reverse phase 1050 HPLC system fitted with a UV detector (Agilent Technologies UK Limited, Stockport, Cheshire, U.K., SK8 3GR). Analytical column: Phenomenex Gemini 5 µm 110A 250 mm x 4.6 mm C18 Silica HPLC Analytical Column (Hichrom, Theale, Berkshire, U.K., RG7 4PE). Column temperature: 45ºC. The injector program was used to first draw 10 µl from the sample vial at undiluted sample strength, and then diluted it with 50 µl of 1% (v/v) orthophosphoric acid in water drawn from a series of vials. This was mixed in the loop and injected. Detector: Multi wavelength UV detector. Signal 1: The sum of signals at 280 and 340 nm via ADC Channel A (ADC1 A). Each signal had a bandwidth of 10 nm. Signal 2: The signal from 260 nm via MWD1 channel bandwidths and reference wavelengths as signal 1. Eight of the 10 compounds were monitored using signal (ADC1 A), with vanillic acid and ellagic acid monitored using signal 2 (MWD1) to give greater sensitivity and selectivity.

2.3 Sensory analysis

2.3.1 Sensory panel

All sensory tests were carried out by Scotch whisky Research Institute's internal sensory panel, which consisted of 22 trained members of staff who had undergone extensive sensory training and had substantial experience in the assessment of whiskies and related products, regular training and sensory test also applied to monitored panel performance. The panellists were introduced to a concept of aroma interaction and trained intensively to peat related notes.

2.3.2 Aroma profiling by Quantitative Descriptive Analysis

Quantitative Descriptive Analysis (QDA) was used to determine differences in the aroma profiles among the whiskies. The attributes tested were the general aromas

listed on the whisky aroma wheel (Figure 1.8), namely: pungent, peaty, feinty, cereal, green/grassy, floral, fresh fruit, dried fruit, solventy, soapy, sweet, woody, spicy, oily, sour, sulphury, stuck match, and stale.

These aroma attributes can be subdivided into the related aroma character attributes as showing in Table 2.7. A total of ten aroma attributes were initially tested with four basic blending element whiskies (Chapter 3.1). For Chapter 3.2 - 3.4 samples set experiments, specific aroma attributes were used to focus on the measurement of the sample related aroma profile characters.

Table 2.7 – Whisky aromas attributes for the QDA test.

Attributes	Aroma character
Floral/Sweet	Fresh flowers, perfumed, vanilla, honey
Fruit/Solventy	Estery : Apples, pears / Paint thinners
Green/ grassy	Fresh herbal, green leafy
Woody	Woody, spices and dried fruit
Sour	Vinegary, cheesy, sickly
Peaty	Burnt, smoky, medicinal
Cereal/Nutty	Malt, nutty, mash-like, cereals, toasted
Feinty	Leathery, tobacco, sweaty, fishy
Sulphury	Rubbery, cooked vegetable, meaty, stagnant
Soapy/oily	Buttery, fatty, rancid, unperfumed soap

2.3.2.1 Sample preparation

For sensory testing, the sample was poured into a tulip shaped nosing glass. A watch glass was placed on top to limit the evaporation of aroma volatiles from the headspace above the spirit. Still water was added to the whisky sample to bring the alcohol content to a common whisky nosing strength, around 20% (v/v) ethanol. Dilution was used to decrease the pungency effect of the ethanol and 'open' the full aroma characteristics of the whisky. Finally, the glass was swirled and the aroma assessed.

2.3.2.2 Test procedures

Overall 27 samples (Table 2.5) were tested in the study. The whole study was split into five sessions, in each session, selected types of whisky samples were tested with selected sensory attributes as showing in Table 2.8 below.

Table 2.8 – Five Chapters whisky aroma profile sensory test and the aroma attributes used in each session (whiskies detail list in **Table 2.5**).

Chapter	Sample types	Aroma attributes tested
3.1	Four basic whiskies	Sweet/Floral, Fruity/solventy, Green/Grassy, Woody, Sour, Peaty, Cereal/Nutty, Feinty, Sulphury, Soapy/Oily
3.3	Standard grain whiskies (x4)	Sweet/Floral, Fruity/solventy, Green/Grassy, Sour, Cereal/Nutty, Feinty, Sulphury, Soapy/Oily
3.4	Woody grain whiskies (x5)	Woody related: Dried fruity, Spicy, Sweet, Overall woody intensity
3.2	Peated malt whiskies (x6)	Peaty related: Burnt, Smoky, Medicinal, Overall peaty intensity
3.3	Unpeated malt whiskies (x8)	Sweet/Floral, Fruity/solventy, Green/Grassy, Sour, Cereal/Nutty, Feinty, Sulphury, Soapy/Oily

Testing samples were placed in individual booths under red colour masking lights at room temperature in coded standard whisky tasting glasses to minimize any influence of sample colour. Panellists were asked to score them in terms of the intensity of each attribute. Scores were given on a line scale of 0 to 3 with intervals of 0.1. For performing QDA, assessors were asked to give a score for each attribute, even if this was zero, to ensure that attribute was not inadvertently omitted. Sensory tests were designed, run and collated using specialist sensory software (Compusense V.5, Compusense Inc., Ontario, Canada). Data were exported to Excel (Microsoft Office 2007) and Unistat 5.0 (Unistat Ltd., London, U.K.) for further analysis. ANOVA was then applied using Compusense to determine if there were significant differences between the sample means.

2.3.3 Aroma interaction studies

Whisky matrices used in blends will potentially interact and mask the peaty character in a blended whisky. This aroma interaction and masking effect in this study are named and defined as Aroma Interaction Capacity (AIC), which can be measured by both a scaling and threshold approach and represented by a numerical relationship.

2.3.3.1 Aroma interaction study using a scaling test

One major requirement of this study was the capability of determining the aroma interaction of the peaty aroma with the blended whisky. Laboratory made blended whisky samples were prepared to explore the impact of blending using the line-scale test (Ebeler 2004).

Sample preparation

In these blending sensory study series, all the samples were made by adding fixed volumes of peated malt (10% (v/v) Caol Ila) to different whisky samples (matrices), to produce blends containing the same level of peated malt. Figure 2.5 illustrates the proportion of whiskies used in the experiment for the studies of aroma interaction during blending.

The laboratory-made blended samples were prepared for two types of analysis purposes, firstly the line-scaling method was used to measuring the aroma interaction capacity for each type of whisky. Such as in Chapter 3.1.1, the line-scaling method was used for scale aroma interaction analysis. This line-scaling method was also used to evaluate laboratory-made blends with more complex compositions, and to evaluate the performance of the prediction model (Chapter 3.5.1).

Figure 2.5 – Example of blending samples preparation percentage make up

Test procedure

The test procedure for the line-scaling analysis was similar to the QDA described in Chapter 2.3.2.1. Samples were identified using three digit random codes, and the presentation order was randomised. Each blended sample was presented to the assessor, who was asked to score them for the overall intensity of peaty character.

Scores were given on a line scale of 0 to 3 with an interval of 0.1. For performing the line scale marking analysis, assessors were required to give a score for each attribute, even if this was zero. Test results were collected and summarized by the same method described in previously.

2.3.3.2 Aroma interaction study by measuring thresholds of phenols (threshold approach 1)

Materials and sample preparation

Eight phenolic compounds (presented in Table 2.1) were measured in this threshold experiment. The test samples were prepared by dissolving individual phenolic compounds into each of the test matrices (unpeated malt, standard grain and woody grain whiskies), all of the 40% (v/v) bottle strength whisky samples were diluted to 20% (v/v) by using distilled water prior to testing.

Table 2.9 – Dilution series of phenolic compounds based on series of geometric sequence at mg/l. 1C are the phenolic compounds threshold in 20% (v/v) ethanol solution obtained from SWRI database (Table 2.1).

Phenolic Compounds	Dilution set					
	1/4 C	1/2 C	1 C	2 C	4 C	8 C
(P1) guaiacol	0.01	0.02	0.04	0.08	0.16	0.32
(P2) 4-methylguaiacol	0.24	0.48	0.95	1.90	3.80	7.60
(P3) *o*-cresol	0.15	0.31	0.61	1.22	2.44	4.88
(P4) phenol	4.80	9.60	19.20	38.40	76.80	153.60
(P5) 4-ethylguaiacol	0.03	0.06	0.11	0.22	0.44	0.88
(P6) *p*-cresol	0.01	0.03	0.05	0.10	0.20	0.40
(P7) *m*-cresol	0.15	0.29	0.58	1.16	2.32	4.64
(P8) 4-ethylphenol	0.12	0.24	0.47	0.94	1.88	3.76

The final phenol concentration was based on a geometric series concentration which was based on the reference odour threshold concentration of phenol in a 20% (v/v) ethanol matrix. An example is shown in Table 2.9. If the concentration 'C' is the phenol detection threshold in 20% (v/v) ethanol (reference from Table 2.1), then the phenol concentration in the set of test solutions will be 0.25C, 0.5C, 1C, 2C, 4C and 8C, being shown in Table 2.9.

Test procedures and sample presentation

Triangle test-alternative forced-choice was used during the assessment, a forced-choice procedure—different from control (Olsson and Cain 2000; Yarnitsky and Pud 2004; Danzer 2007) was also applied to minimize the anticipation effects (eliminating a false positive response). The difference from control (DFC) sample presentation was similar to the paired comparison test in that each sample was compared with a reference control sample (Ahmed et al. 1978).

Sample display

During the test, panellists were given six glasses of samples containing increasing concentrations of phenols. Glasses were coded alphabetically from A (the lowest) to F (the highest) and presented in ascending order. 'Control' sample (without phenol) was included for the comparison purposes and each phenol-containing sample was assessed as being similar to or different from the control sample (DFC). The assessors were asked to designate the first phenol contained sample in which they could detect difference from the control sample. The option of 'all the same' was given to assessors if they could not detect a difference from the control in any of the six samples tested. The test samples were deliberately presented in ascending concentrations order (Gregson 1962). This phenols threshold test was carried out only by nosing due to the food grade phenols not being availability during the period of this study.

Threshold calculation

The statistical analysis for determining the threshold values involved predicting the concentration that corresponded to 50% correct responses. As shown in Figure 2.6, the *x*-axis represents the concentration of the stimulant; the *y*-axis represents the proportion of correct responses, with a fitted curve. By using the logarithms to base 10 with the response curve to give linear relationship $Y = aX + b$. The threshold is determined by the value of concentration that corresponds to a response level of 50% (Lawless and Heymann 1998b).

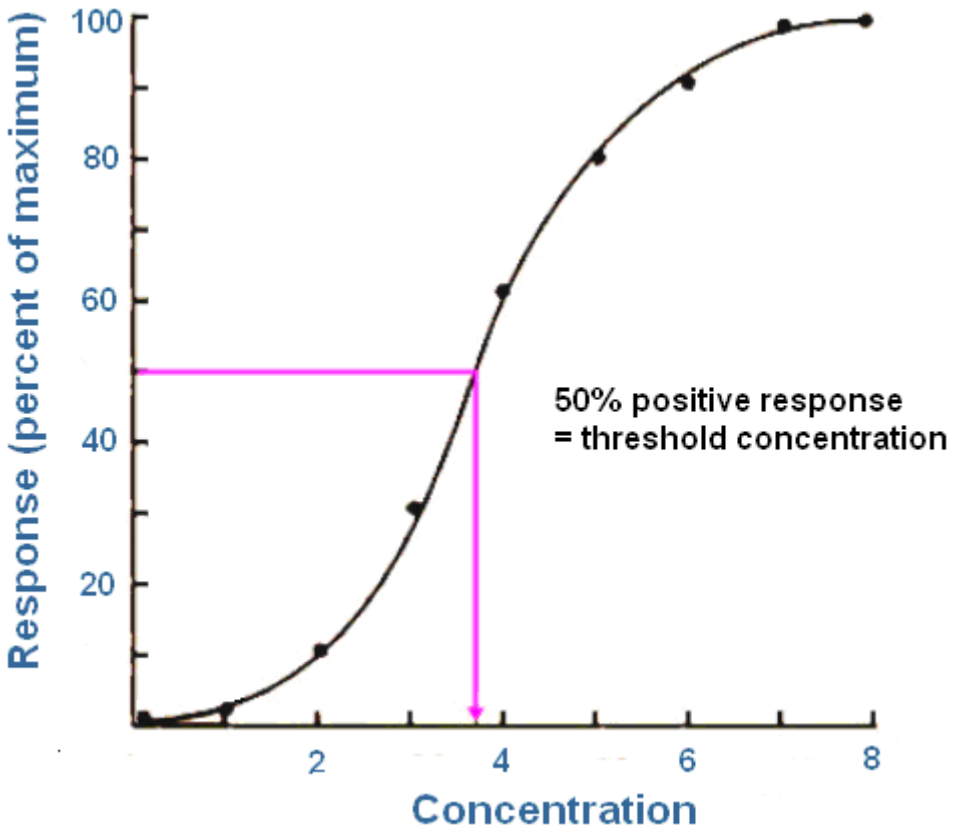


Figure 2.6 – Idealised frequency of correct responses for stimulus detection as a function of physical intensity (*ie* concentration) forms a psychometric function that resembles the S-shaped curve of the cumulative normal distribution, and logarithms to give a linear relationship (Lawless and Heymann 1998b).

2.3.3.3 Aroma interaction study by measuring thresholds of peated malt (threshold approach 2)

Sample preparation

The test samples were prepared by combining various volumes of peated malt (Caol Ila) with standard grain, woody grain and unpeated malt whisky. The peated malt concentration used was based on a series of ascending sequence, 0.5%, 1%, 2%, 4%, 8%, 16% (v/v). The volume range used in the threshold test was pre-tested by a selected group of panellists (8-16 people). All of the 40% (v/v) bottle strength samples were reduced to 20% (v/v) using still water before testing.

Test procedures and threshold calculation

The peated malt threshold test was carried out using the same method used for the phenols (Chapter 2.3.3.2).

2.4 Aroma interaction study using potent aroma marker compounds

2.4.1 Identification of the potent aroma marker compounds

Solvent extraction followed by concentration method in connection with (GC-O-AEDA) Gas Chromatography – olfactometry-aroma extract dilution analysis was used to determine the potent odorants in the different types of whiskies. A typical GC-O test obtained GC chromatography obtained for unpeated malt is shown in Figure 2.7.

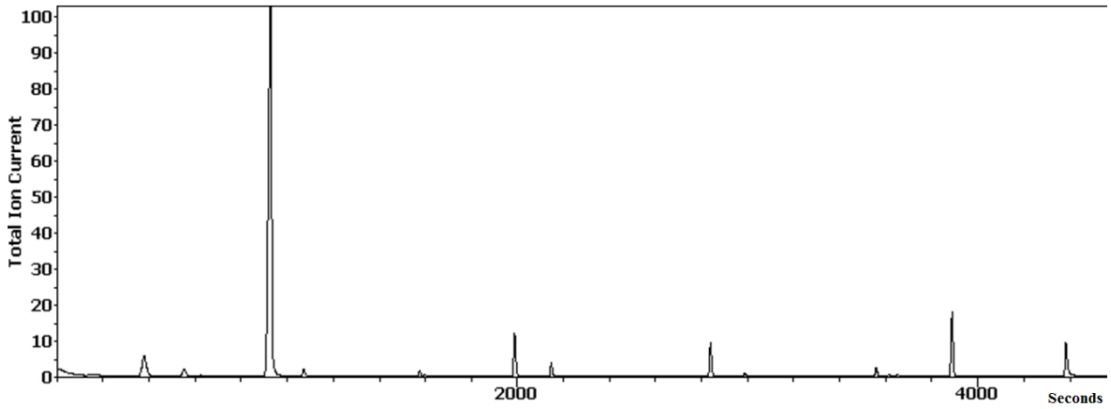


Figure 2.7 – Typical GC-O test chromatogram produced from an unpeated malt sample GC-O analysis screenshot.

Analytical instrumentation

Analyses were carried out by GC-MS (HP 6890/5973 GC-MS, USA) and coupled to the Phaser Sniffing Port OP275 (ATAS GL International BV, Veldhoven, Netherlands). The column used was a 60 m x 0.32 mm DB-Wax capillary column with a film thickness of 1 μm. The carrier gas was helium at a flow-rate of 2.2 ml min^{-1}. The initial oven temperature was 40°C, held for 1 minute, increasing to 250°C at 7°C min^{-1} with a final holding time of 5 min. The injector temperature was maintained at 240°C with splitless injection model. The mass spectrometer was operated in the electron impact (EI) 70 eV mode and ions from 35 to 400 amu were scanned at a rate of 2 scans s^{-1}.

A four-port splitter stand was located in the GC oven; two ports were connected to the analytical column outlet and an auxiliary gas outlet; the two remaining ports were connected to the MS and the transfer line (sniffing port) by means of retention gaps. The transfer line, kept under constant temperature of 250 °C, terminates in an ergonomic glass nose cone. An auxiliary gas (helium) flow of 1 ml/min was maintained constantly during analyses. Data were collected by Thumb level switch

software (Software Workstation).

In order to maximise aroma detection and prevent vapour condensation before it reached the nosing port, the transfer pipe was heated so that the air flow with the same temperature as GC oven. The nosing port was equipped with a humidifying device to prevent the loss of sensitivity due to dry nasal mucosa.

The chemical composition of the identified odour compound was then checked by mass spectra with those of the NIST libraries stored in the data system, by comparing with published mass spectra or by interpretation of fragmentation patterns. Then the pure form of the recommended chemical compound was further evaluated by GC/MS-O by direct injection for its characteristics such as aroma character, intensity, mass spectrum and chromatographic properties i.e. retention index (RI). The obtained data was then compared and matched with the characteristics of the identified odour. The identification of this key compound was therefore confirmed.

Whisky sample preparation

Three typical Scotch whisky samples were tested in this study (unpeated malt, standard grain and woody grain). In order to perform the ADEA test, a liquid-liquid extract was applied to concentrate the whiskies. Samples were prepared by gently shaking (each 100 ml whisky sample) for 10 min with 20 ml n-pentane in a 200 ml separating funnel. The n-pentane layer was then collected and concentrated to around 5 ml by nitrogen blowing over the samples in a water bath set at 32°C (pentane, boiling point 36°C).

In order to perform AEDA with GC-O instrument, the collected pentane solutions were further diluted down to three different concentrations 1/1 (non-dilution), 1/10 (10 times dilution) and 1/100 (100 times dilution) (Blank 1996; Poisson and Schieberle 2008). Therefore, 1 ml of extracted sample (pentane solution) was place into 10 ml and 100 ml volume flask and diluted with ethanol. Three samples and their three dilutions (overall 9 samples) were assessed by GC-O over different days. GC-O AEDA test was carried out on each sample by four members of the SWRI sensory panel.

GC-O assessors

Four experienced sensory assessors, all members of the SWRI Sensory Panel, were involved in this study (one female and three male), who had undergone extensive sensory training and had substantial experience in the GC-O assessment. All the panellists were introduced to a concept of aroma extract dilution analysis and trained for using GC-O data collection software. When a volatile compound was detected at least three times, this analyte was then declared as a confirmed identified aroma compound. Four panellists assessed each sample. There two types of detection odour categories been recorded.

- The total detected odours, means all the odours been detected by any of the panellists during the GC-O test were accounted.
- The total confirm identified odour, which indicated the odour have been commonly detected by at least three panellists (majority of the panellists in four).

GC - Olfactometry operation principle

For our instrumental configuration, many aroma-active compounds have a chromatographic duration time short than 2 seconds. As a consequence, it appeared unrealistic to expect an instant and systematic feedback in a GC-O run, because the panellist is likely to have insufficient time to react and write down before each stimulus ends. Therefore the commonly used aroma vocabulary (Chapter 2.3.2) was pre-programmed into the olfactometry software system in advance. During the GC-O test, the panellists can select the related aroma vocabulary to define the detected aroma. Panellists were instructed to keep the mouse button depressed until the detected aroma disappeared for recorded the duration time. During this period, panellists can also select the intensity of this aroma by clicking the right mouse button (Computer pre-setup intensity, no click-weak, one click-medium, two clicks-strong).

2.4.2 Determination of the aroma interaction capacity of the marker compounds

In this test the potent marker aroma compound were studied used as matrix background solutions. The aroma interaction capacity of these markers was evaluated

by determining the sensory panels' ability to smell peated malt in these solutions using a threshold approach.

2.4.2.1 Sample preparation

Individual solutions were prepared of each marker compound at 5times of their sensory threshold levels in 20% (v/v) ethanol. From these are series of glasses were prepared containing these solutions plus 0.5%, 1%, 2%, 4%, 8% (v/v) peated malt (labelled A-E). A control sample was also prepared containing no peated malt.

2.4.2.2 Test procedures and threshold calculations

Assessment was carried out in three sessions (with one marker compounds in each section), and each test was repeated on a second day. Panellists were asked to compare each of the test samples with the control starting from A (with the lowest peated malt content) through to E (the highest) and to designate the first sample in which they could detect the peaty character. The option of 'cannot detect peaty character at all' was given to assessors if they could not detect a difference from the control in any of the five samples tested. The test samples were deliberately presented in ascending order (concentrations) as recommended (Gregson 1962) to prevent fatigue from carryover of higher concentrations of aromas. This sensory test was carried out only by nosing due to the potential harmful from pure chemical solution, which making them unsuitable for taste tests. Thresholds were calculated following the procedure previously outlined in Chapter 2.3.2.2.

CHAPTER 3. RESULTS AND DISCUSSION

The primary objective of this study was to determine how the composition of a blended whisky affects the perception of aroma. In principle there could be many different types of aroma interaction that occurred when tasting whisky, such as taste-smell interaction, influence of temperature, colour, texture, sound and different diluents (Delwiche 2004). The majority of such interactions have been identified by the changes in chemical composition. However, the understanding of how these changes effect the sensory attributes in whisky is lacking, due to the complicated nature of the aroma in the whisky matrix. Although the general problems in the field of interactions are known, very little experimental data has been published, and the mechanism behind the science of the aroma interaction remains unclear, which hinders any predicting assumptions. Jounela-Eriksson (1983) has observed both synergistic and suppressive effects on imitation whisky solutions by observing variation in thresholds level (Salo 1973). Meilgaard *et al.* (1971) made early attempts to characterize the aroma interactions between aroma compounds in beer. The compounds were studied in the mixtures of varying complexity, imitating the composition of the beer. Conner *et al.* (2003b) have identified the maturation related compounds that participate in the interactions and these involve some degree of the overall aroma suppression of whisky. Conner *et al.* (1998) have found the presence of the surface active distillate components, such as long-chain alcohols, aldehydes, esters and dissolution of wood extractives caused a number of changes in the solubility parameters of organic compounds, which could result in lower headspace concentrations, and thus have a direct effect on the aroma of the matured spirit. Sterckx *et al.* (2011) observed various aroma interactions in binary mixtures of eleven monophenols, ranging from partly additive to strong synergistic for all combinations of monophenols, except for one combination showing suppression.

The ultimate aim of this study is to examine the aroma interactions between different components in a blend and their effect on the threshold and perception of individual aromas. An attempt is made to verify statistically all observed aroma interaction and to explain the factors producing these interactions, and their effects on the total intensity of aroma complex. This study mainly focused on the aroma-aroma interaction in relation to peaty character in whisky and the response behaviour of such

interaction corresponding to the sensory perception of the blended whisky.

3.1 Perception of peaty character and its relationship with matrix aroma interaction, chemical composition and sensory character

This first part of this study examined the impact of three whisky matrices, namely unpeated malt, standard grain and woody grain (described in Chapter 1.2.6 and sample prepared from basic whiskies listed in Table 2.5) on the perception of peaty character, in the form of added peated malt (Caol Ila Table 2.5). The whisky matrices were characterised in terms of their composition and original sensory character. The collated analytical and sensory data were then assessed using statistical analysis, to explore the properties of three individual whisky matrices and their related aroma interaction capacities.

3.1.1 Analytical and sensory comparison of the three whisky matrices and of the peated malt

In this section, the three base whisky matrices were compared using a number of analytical and sensory techniques. The peated malt samples were similarly characterised.

3.1.1.1 Phenolic compounds

Table 3.1 – Mean (three analyses) concentrations of headspace phenolic compounds (mg/L) with % RSD for grain and malt whiskies (p-values at 5% significance level).

Phenolic compound	Grain whisky		Malt whisky		p-value
	Standard	Woody	Unpeated	Peated	
	Mean (%RSD)				
(P1) guaiacol	0.03 (0.9)	0.04 (2.8)	0.03 (0.9)	1.18 (15.8)	<0.05
(P2) 4-methylguaiacol	0.04 (1.1)	0.04 (1.1)	0.04 (0.4)	0.50 (12.0)	<0.05
(P3) *o*-cresol	0.05 (1.0)	Not detected	0.06 (1.2)	1.64 (13.7)	<0.05
(P4) phenol	0.03 (8.2)	0.03 (5.1)	0.03 (2.7)	2.19 (14.4)	<0.05
(P5) 4-ethylguaiacol	0.05 (3.4)	0.04 (3.4	0.04 (0.4)	0.61 (7.2)	<0.05
(P6) *p*-cresol	0.05 (0.4)	0.05 (1.3)	0.04 (2.5)	1.40 (15.0)	<0.05
(P7) *m*-cresol	0.04 (1.2)	0.04 (0.1)	0.04 (0.6)	0.45 (11.0)	<0.05
(P8) 4-ethylphenol	0.05 (1.6)	0.05 (0.2)	0.05 (1.4)	0.72 (14.1)	<0.05
Total phenol	0.4	0.2	0.3	8.7	<0.05

The level of phenolic compounds in the headspace of each of the whiskies is shown in Table 3.1. As expected the peated malt contained the highest levels of phenolic compounds compared to the other whiskies due to the peat-kilning process. However, a small amount of phenols was also detected in other whiskies. This might be explained by the formation of the other sources of the phenolic compounds during fermentation and maturation (Steinke and Paulson 1964; Jounela-Eriksson and Lehtonen 1981; Paterson and Piggott 1989; Beek and Priest 2000).

3.1.1.2 Major distillate congeners

Levels of the major distillate congeners in the three whisky matrices and in the peated malt are shown in Table 3.2. The unit of major distillate congeners g/100L is based on the measurement units comments used by Scotch industry (Aylott 2003).

Table 3.2 – Mean (three analyses) concentrations (g/100 L) of major distillate congeners with % RSD for grain and malt whiskies (p-values at 5% significance level).

Major congener	Grain whisky		Malt whisky		p-value
	Standard	Woody	Unpeated	Peated	
	Mean (%RSD)				
(D1) acetaldehyde	2.68(3.6)	3.18(18.8)	7.90(1.8)	7.00(10.0)	<0.05
(D2) ethyl acetate	18.58(4.4)	22.78(3.4)	26.18(14.1)	27.40(3.5)	<0.05
(D3) acetal	1.45(4.0)	1.85(24.0)	4.23(3.6)	3.88(12.2)	<0.05
(D4) methanol	10.78(17.5)	11.65(1.5)	4.65(1.2)	4.68(6.8)	<0.05
(D5) n-propanol	74.40(10.2)	62.50(2.4)	46.85(8.6)	47.05(5.7)	<0.05
(D6) iso-butanol	50.68(5.5)	68.80(3.6)	53.73(9.5)	71.18(6.2)	<0.05
(D7) iso-amyl acetate	1.00(31.6)	1.18(38.9)	1.60(8.8)	3.33(15.0)	<0.05
(D8) n-butanol	0.00	0.00	1.40(5.8)	1.65(10.5)	<0.05
(D9) 2-methyl-1-butanol	2.55(3.9)	3.55(3.6)	41.28(15.5)	58.45(7.2)	<0.05
(D10) 3-methyl-1-butanol	6.33(7.2)	8.08(3.7)	121.98(10.4)	144.60(7.0)	<0.05
Total major congeners	168.6	183.8	309.9	369.4	<0.05

Table 3.2 shows the significant differences in all major congeners between the whisky types ($p < 0.05$), with the difference mainly being between the grain and malt whiskies (malt > grain). This would be expected due to the different distillation processes used in their production.

3.1.1.3 Trace distillate congeners

The levels of trace congeners in each whisky type are shown in Table 3.3. The unit of trace distillate congeners mg/L is based on the measurement units comments used by Scotch industry (Aylott 2003).

Table 3.3 – Mean (three analyses) concentrations of trace congener (mg/L) with % RSD for grain and malt whiskies (p-values at 5% significance level).

Trace congener	Grain whisky		Malt whisky		p-value
	Standard	Woody	Unpeated	Peated	
	Mean (%RSD)				
(D11) ethyl hexanoate	0.00	0.00	1.73(8.3)	1.30(5.2)	<0.05
(D12) ethyl octanoate	0.75(1.1)	0.28(13.9)	12.53(2.1)	9.32(0.4)	<0.05
(D13) ethyl decanoate	1.66(50.8)	0.50(68.9)	26.23(4.2)	24.46(2.8)	<0.05
(D14) ethyl dodecanoate	1.56(0.6)	0.49(1.9)	9.05(1.1)	14.78(0.4)	<0.05
(D15) ethyl tetradecanoate	0.54(1.3)	0.14(2.1)	0.80(0.6)	1.30(0.8)	<0.05
(D16) ethyl hexadecanoate	2.23(0.4)	0.44(1.0)	0.74(1.6)	1.36(1.2)	<0.05
(D17) ethyl 9-hexadecenoate	0.67((3.5)	ND	0.72(3.7)	2.83(2.2)	<0.05
(D18) 2-phenethyl acetate	0.24(15.1)	ND	2.31(2.0)	5.53(3.3)	<0.05
(D19) 2-phenethyl alcohol	1.72(0.5)	1.55(74.6)	21.78(3.0)	24.65(1.3)	<0.05
Total trace congeners	9.4	3.4	75.8	85.9	<0.05

The results showed differences between the two main whisky types (malts and grains), for all trace congeners. Most of these trace congeners are esters that consist of a long chain structure. The quantity and the relative proportion of the esters in a whisky are of the greatest importance for the overall aroma perception because the concentration of the esters in malt is generally above the threshold (Table 2.3).

3.1.1.4 Maturation-derived compounds

The levels of maturation derived compounds in the four whisky samples are shown in (Table 2.4). The woody grain whisky contained by far the highest levels of all of these compounds, with a total amount of maturation congeners of over 30 mg/L. Although all four whiskies had been matured for the same length of time (Table 2.5), namely three years, the woody grain had been held in first-fill ex-bourbon casks. The other whisky types contain much lower concentration of maturation-related compounds because they had been matured in refilled bourbon casks where the wood-

derived congeners have already been partially extracted during previous fills (Chapter 1.5.2.1).

Table 3.4 – Mean (three analyses) concentrations of maturation-derived compounds (mg/L) with % RSD for grain and malt whiskies (p-values at 5% significance level).

Maturation-derived compounds	Grain whisky		Malt whisky		
	Standard	Woody	Unpeated	Peated	p-value
	Mean (%RSD)				
(W1) gallic acid	2.31(4.3)	3.42(3.1)	0.64(11.7)	1.05(4.4)	<0.05
(W2) ellagic acid	4.77(0.6)	10.91(0.1)	2.73(2.2)	2.60(1.6)	<0.05
(W3) coniferaldehyde	0.42(4.8)	2.32(0.9)	0.31(5.7)	0.28(6.9)	<0.05
(W4) vanillin	1.29(1.8)	3.57(1.1)	1.40(3.7)	1.03(2.4)	<0.05
(W5) vanillic acid	0.65(6.8)	1.43(1.9)	0.66(8.7)	0.51(5.5)	<0.05
(W6) sinapaldehyde	0.44(8.4)	1.88(1.1)	0.16(22.8)	0.24(25.8)	<0.05
(W7) syringaldehyde	2.53(4.5)	9.57(0.6)	2.55(3.9)	1.95(2.5)	<0.05
(W8) syringic acid	0.96(5.0)	2.51(4.1)	1.19(2.0)	1.23(10.6)	<0.05
(W9) scopoletin	0.30(1.1)	1.06(7.9)	0.34(11.2)	0.20(2.2)	<0.05
(W10) 5-HMF	0.50(1.4)	2.20(0.1)	0.21(5.1)	0.15(29.6)	<0.05
Total	14.2	38.9	10.2	9.3	<0.05

3.1.1.5 Comparison of aroma profiles

Quantitative Descriptive Analysis (QDA) was used to produce aroma profiles for each of the whisky samples. The mean panel scores are shown in (Table 3.5).

Table 3.5 – Mean QDA scores for aroma attributes of each whisky type and ANOVA, using whisky types as a factor

Aroma Attribute	Standard Grain	Woody Grain	Unpeated Malt	Peated Malt	p-value
Sweet/Floral	1.1	1.3	1.3	0.6	0.027*
Fruity/Solventy	1.1	1.0	1.5	0.3	0.001*
Green/Grassy	0.8	0.9	1.2	0.6	0.148
Woody	0.9	1.8	1.2	0.9	0.011*
Sour	0.6	0.6	1.2	0.4	0.006*
Peaty	0.7	0.3	1.1	2.4	0.011*
Cereal/Nutty	0.9	0.6	0.9	0.8	0.538
Feinty	0.8	0.5	0.7	0.6	0.282
Sulphury	1.0	0.2	0.8	0.8	0.011*
Soapy/Oily	0.7	0.3	0.9	0.6	0.116

**p < 0.05 indicating a significant difference of aroma attributes between the whisky types*

Table 3.5 shows that the majority of the aroma attributes are significantly different between the whisky types ($p < 0.05$), except for Green/Grassy, Cereal/Nutty, Feinty and Soapy/Oily.

The attributes that showed significant difference are discussed below according to their sensory data:

1. Sweet/Floral: the difference for this attribute was mainly between the peated malt and other three whiskies matrices, as the score for Sweet/Floral was much lower in peated malt.
2. Fruity/Solventy: again the significant difference was mainly due to low scores for the peated malt.
3. Woody: as expected the woody grain scored highest for woody character.
4. Sour: the difference for sour was mainly due to higher score for the unpeated malt.
5. Sulphury: the difference for sulphury was mainly due to lower score for the woody grain.
6. Peaty: as expected the peaty character score were much higher in peated malt.

For better comparison and understanding, the QDA scores are presented as a radar plot (Figure 3.1).

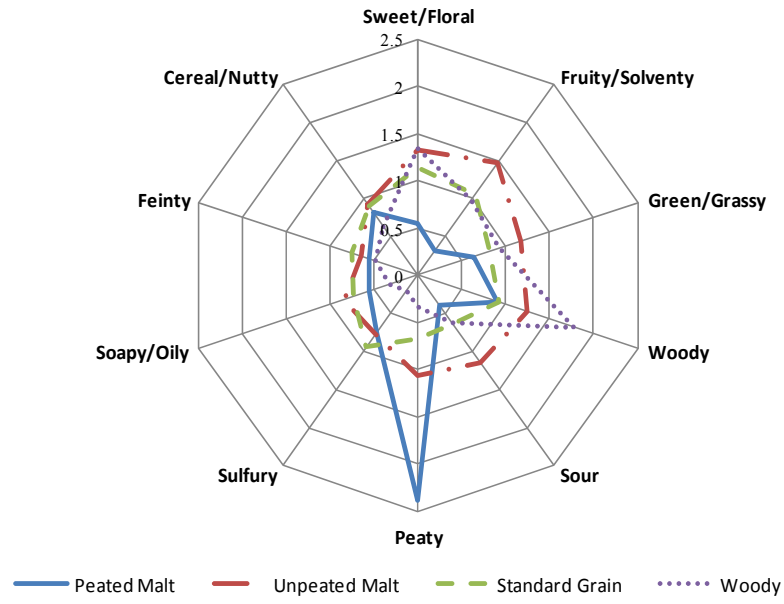


Figure 3.1 – Radar plot of aroma attributes for the basic Scotch whisky types.

The first impression observed from Figure 3.1 is the expected peaty character clearly demonstrated by the peated malt. Whereas, with the exception of peaty character, peated malt is lower in almost all other aroma attributes, particularly sweet/floral, fruity/solventy, green/grassy, sour and woody characters. This phenomenon conflicts with the previous chemical profile analysis (Chapter 3.1.1), which showed that the malt whisky (i.e. unpeated and peated malts) contained higher distillation-related compounds than the grain whisky i.e. standard and woody grains. This anomaly might be explained by the physiological interactions of aroma (i.e. matrix or aroma interaction). As a result, the perceived aroma attributes in peated malt were largely suppressed by the intense heavy peaty aroma (Figure 3.1). This particular pattern of the multi-aroma interaction is also observed for the woody grain sample. A strong woody aroma is a dominant part of the aroma profile in the woody grain. There is simultaneous suppression of other aroma characters such as sulphury and peaty attributes. It is also likely that the more active cask will have an enhanced ability to reduce the intensity of sulphury character (Conner et al. 2003).

In comparison, the unpeated malt and the standard grain had relatively balanced aroma profiles with each attribute evenly distributed. This can be explained by the absence of any dominant aromas. Both the unpeated malt and standard grain samples used in this part of the study were vatted whiskies, which were made by blending whiskies obtained from different distilleries. The vatted whiskies have more balanced overall character compared with products from a single distillery (Conner et al. 2003).

3.1.1.6 Relationships between composition and aroma in whisky matrices

Principal Component Analysis (PCA) was carried out on the combined analytical and sensory data for the three whisky matrices and the unpeated malt. The first two components explained 86.77% of the total variance. The scores and loadings on these components are shown in **Error! Reference source not found.** and **Error! Reference source not found.**.

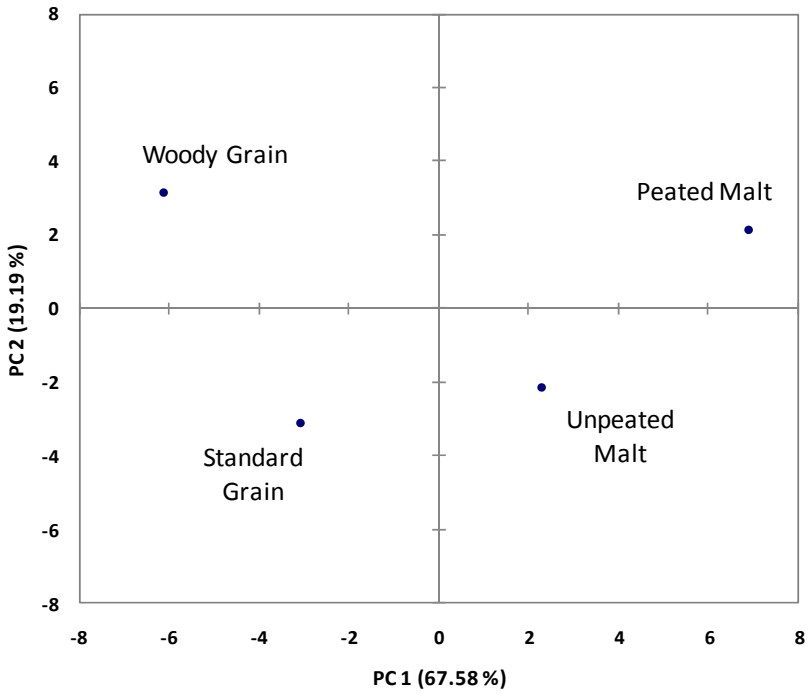


Figure 3.2 – Score plot for the PCA of the combined analytical and sensory data.

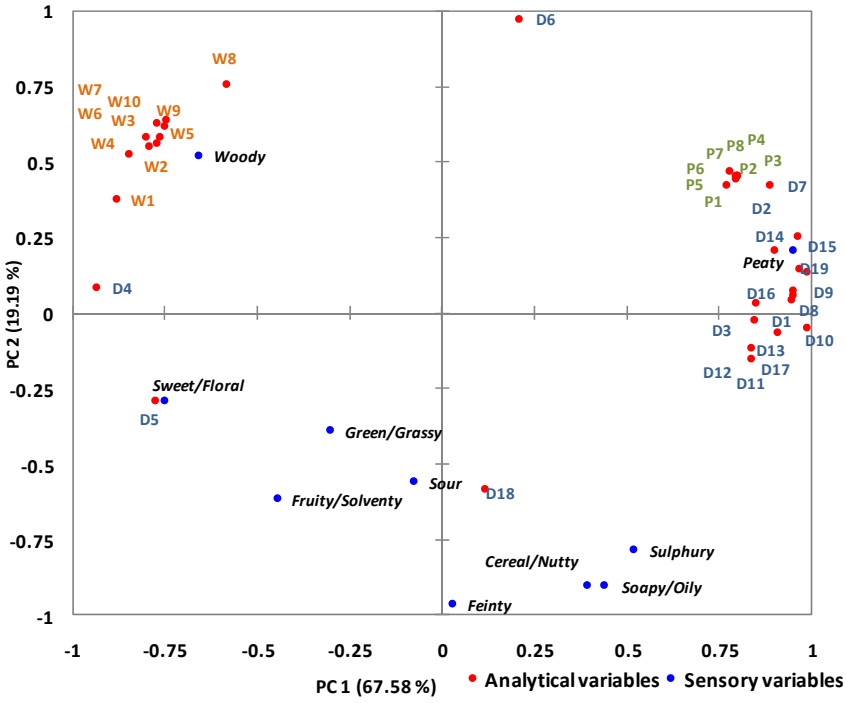


Figure 3.3 – Loadings plot for the PCA of the combined analytical and sensory data. D (Distillate character congeners), P (Peaty character congeners), W (Woody character congener).

In Figure 3.2, each of the whisky types is situated in individual quadrants. The malt whisky type is located on the right hand side of the plot with positive PC values, and

the grain whisky type is located on the left hand side of the plot with negative PC values. This can be simply explained by the differences in the analytical variables shown in Figure 3.3, where (D) represents distillate congeners, (W) woody compounds and (P) phenolic compounds and the sensory attributes are shown in full. Here the woody and the peaty aroma characters are mainly associated with the woody grain and peated malt whisky types respectively.

The first PC separates malt and grain spirits, with contribution from trace and major distillate congeners in the linear combination of variables. The second PC resolves on the basis of the presence of other components, such as the maturation derived compounds (woody) and the phenolic compounds (peaty) are clustered in the positions corresponding to the woody grain and peated malt whisky types, respectively. Almost all the major and trace distillate congeners are also found to have an association with the peated malt. Two distillation congeners, D4 (methanol) and D5 (*n*-propanol) were found to be positioned in the upper left quadrant and the lower left quadrant, corresponding to the grain types, respectively. It was general found that the methanol and propanol level are general higher in grain than malt whisky (Table 3.2).

3.1.2 Evaluation of aroma interactions in different whisky matrices

The Aroma Interaction Capacities (AICs) of the three whisky matrices (unpeated malt, standard grain and woody grain) were determined using a scale approach and tests measuring the threshold levels of both phenols and peated malt (threshold approach), as described in Chapters 2.3.2.1, 2.3.2.2 and 2.3.2.3 respectively.

3.1.2.1 Aroma interactions using a scaling test

In this test 10% (v/v) peated malted (Caol Ila) of peated malt was added separately to ethanol 40% (v/v), standard grain, woody grain and unpeated malt. The 10% (v/v) peated malt level was selected to ensure its perception and be picked by the majority of panelists (50% population), but avoid dominance in the blends. Ethanol was used as a reference matrix being expected to have the least aroma interactions during blending. Samples were initially assessed by sensory panel for the main peaty-related

sensory attributes (overall peaty intensity, burnt, smoky and medicinal). The scaled scores (means) for each attributes were recorded (Table 3.6).

Table 3.6 – Mean panel scores of peaty-related aroma attributes for blends with 10% (v/v) Caol Ila blended samples and ANOVA for each attribute, using whisky matrices as a factor.

Sample matrix	Aroma attribute (mean score)			
	Overall peaty intensity	Burnt	Smoky	Medicinal
Ethanol	1.7	1.3	1.4	0.9
Standard grain	1.1	0.9	0.9	0.8
Woody grain	0.6	0.3	0.4	0.5
Unpeated malt	0.5	0.3	0.3	0.4
p-value	0.0001*	0.0001*	0.0001*	0.0013*

**p-values < 0.05 showing the significant differences of the aroma attributes between the whisky matrices*

Table 3.6 shows the scaled scores for the peaty-related aroma attributes for each sample matrix. With the introduction of the same concentration of peated malt, the results showed an evidence of significant difference of the peaty-related aroma attributes between each of the sample matrices ($p < 0.05$). This demonstrates that different blend matrices have a significant impact on perceived peaty character.

Ethanol showed the highest score in the overall peaty intensity due to the occurrence of the least aroma interactions and is the simplest nature of the matrix. Correspondingly, standard grain is a more complicated matrix than ethanol and therefore gives significant lower peaty intensity in score, due to more aroma interaction generated that affect the perception of peaty aroma. Woody grain and unpeated malt matrices are both heavily aromaed whisky types and thus, showed an even lower score for peaty intensity as well as other peaty-related attributes. This implies that woody grain and unpeated malt matrices have the greatest aroma interaction capacities. The results showed the existence of the interactions during whisky blending that affect the degree of peaty aroma perception, with aroma perception influenced by different matrix backgrounds.

Correlations between the aroma attributes were also examined. It was found all three peaty related character medicinal, burnt and smoky are highly significantly ($p < 0.05$)

correlated with the overall peaty intensity. The main reason for the strong correlations between these aroma characters and the overall peaty intensity is that the blends were prepared based on the same peaty aroma source (10% (v/v) Caol Ila). Also, the four attributes tested in this experiment were all peaty character related and derived. To facilitate the efficiency and to simplify the experimental design, it was therefore decided to use only overall peaty intensity as the main parameter to represent the peaty character in all future scaling aroma interaction experiments next.

The scaling method is a relatively quick and easy technique, however, just like other sensory methodology it has limitations (Meilgaard et al. 1999). Scaling methods are considered to be subjective and qualitative, and only provide information on the relative relationships between stimuli of different intensities (Lawless and Heymann 1998b). They do not use a standard stimulus in an attempt to get subjects to make judgments high in absolute accuracy, but try to encourage subjects to use similar comparison scales in order to make results easier to interpret. For this reason, threshold measurements were also carried out in this study, as a more quantitative means of determining differences in aroma interaction capacities.

3.1.2.2 Aroma interactions using threshold methods

In this study, the threshold was used as an alternative approach to study the aroma interactions. In a measurement, the threshold level might be affected by many factors, such as types of substances used, testing environment, ages and health conditions of the panellists etc. (Meilgaard et al. 1999). These interferences could cause substantial variation in the threshold levels obtained, and are normally considered as a potential disadvantage for the threshold measurement. In particular, the interference caused by the background phase (food matrix difference) may encounter a problem, since the release of volatile substances from the matrix into the region of sensory receptors will be dramatically affected by different background matrices used (Malnic et al. 1999). If the peaty aroma is largely influenced by different matrices in a blend, the threshold for peaty aroma would be expected to be influenced by the matrix background. The threshold method used in the study was to evaluate the variation in threshold values caused by different matrix backgrounds, rather than determining the stimulus threshold. Two approaches were used. The first examine the thresholds of added

phenols, while the second compared thresholds of peated malt.

Threshold method - phenols

As mentioned in Chapter 1.2.2.3, it is widely recognized by the Scotch whisky industry that the phenolic compounds are considered as a primary marker for the peaty-related aroma (Lehtonen 1983a; Lehtonen 1983b). Threshold levels of the eight major phenolic compounds were measured for three basic whisky matrices (standard grain, unpeated malt and woody grain) and ethanol. Results are shown in Table 3.7. Samples were present in ascending order to prevent fatigue from carryover of higher concentrations of aromas.

Table 3.7 – Absolute aroma threshold levels (mg/L) of phenolic compounds for standard and woody grain and unpeated malt whiskies (20% (v/v) ethanol).

Phenolic compound	Absolute threshold level (mg/L)			
	Ethanol*	Standard grain	Woody grain	Unpeated malt
4-ethylguaiacol	0.11	0.09	0.16	0.27
4-ethylphenol	0.47	0.45	0.87	0.48
guaiacol	0.04	0.05	0.08	0.10
m-cresol	0.58	0.65	0.93	1.00
4-methylguaiacol	0.95	1.09	1.91	2.83
o-cresol	0.61	0.35	0.49	0.93
p-cresol	0.05	0.03	0.06	0.05
phenol	19.2	17.53	45.57	37.30

**Ethanol threshold obtained from SWRI database Table 2.1*

It can be seen in Table 3.7 that ethanol and the standard grain showed lower threshold levels for all phenolic compounds than the woody grain and the unpeated malt matrices. As previously mentioned, Saison et al. (2009) believed that these compounds can also counteract each other, which is called antagonism, or they can interact positively with each other, displaying a synergistic effect. Based on these results, it is believed that the threshold levels of the phenolic compounds were certainly affected by the matrix backgrounds and this effect could be antagonistic.

It is also important to note that, in a blended whisky, not all of the phenolic compounds can occur at high enough levels to impart a distinctive aroma. For example, phenol is normally present in the highest concentration (Howie and Swan 1984), but it is present only at sub-threshold levels (threshold approximately 19 mg/l)

and is not itself of sensory significance whereas the aroma potential of some other phenols, particularly *p*-cresol and guaiacol, may be greater (Steele et al. 2004).

Each aroma compound can exhibit its aroma character independently; given that each compound should be presented or existed at its threshold level for human perception. It was suggested by Guadagni *et al.* (1963) that the chemically similar compounds might exhibit an additive effect with each other, but they would not be expected to behave in the same way if they have vastly different chemical structures. This is supported by the results shown in Table 3.6, which show that different phenolic compounds interact differently with different matrix backgrounds, implying different degrees of aroma interaction capacity (AIC). For example, in Table 3.7 some compounds such as guaiacol, phenol, *p*-cresol and 4-ethylphenol were suppressed more in the woody grain matrix, whereas other compounds (4-ethylguaiacol, *m*-cresol, *o*-cresol and 4-methylguaiacol) were influenced more by the unpeated malt matrix.

It can be concluded that by using the threshold technique, it might be possible to assess the aroma interaction capacity during blending. However, this study had two main drawbacks:

1. Using phenolic compounds as a stimulus cannot fully represent the entire peaty character (Chapter 1.2.2.3). The data will only provide limited understanding of the aroma interaction on peaty character.

2. Threshold measurement requires intensive labour input and is time-consuming. For one matrix background, a total of eight individual measurements were needed for each compound. This consequently increases the difficulty and the complexity.

Therefore peated malt was used as an alternative stimulus to represent the 'real' peaty character and simplify the testing procedures.

Threshold method - peated malt

Threshold testing was repeated using peated malt as a stimulus in the three basic whisky matrices and ethanol. In this case, the recognition threshold was examined (Chapter 1.5.2.2), i.e. the level at which the sample had recognizable peaty characteristics. Results are shown in Figure 3.4.

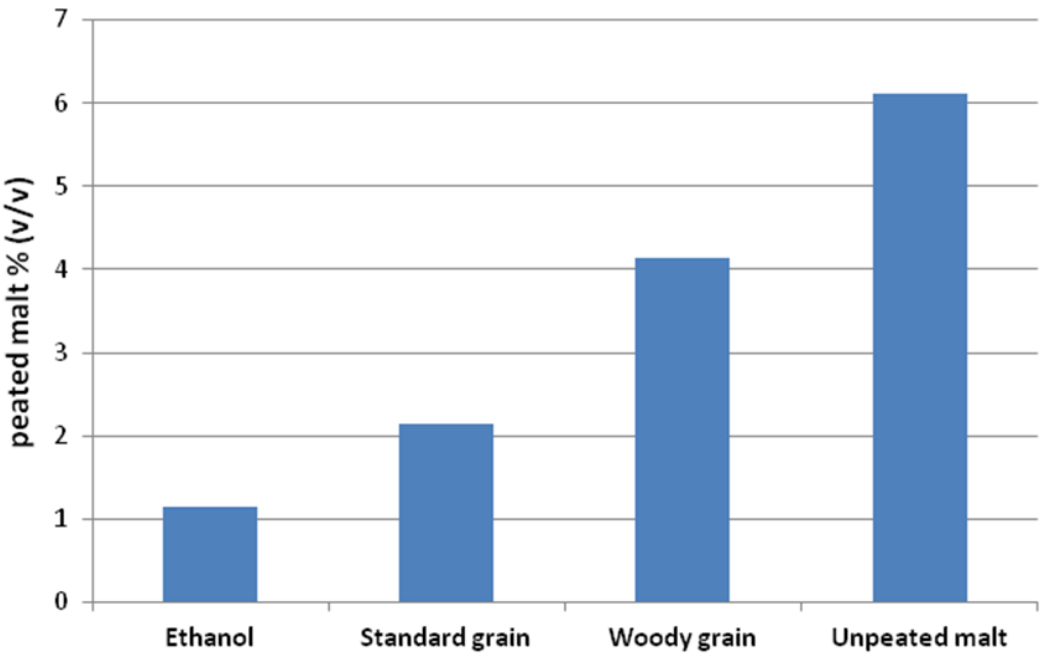


Figure 3.4 – Recognition threshold levels of peated malt for four basic whisky matrices.

In Figure 3.4, an increasing trend for the threshold levels was observed relating to increasing complexity of the matrix nature (ethanol > standard grain > woody grain > unpeated malt). This agreed with the previous observations made using the scaling test (Table 3.6), where the overall peaty intensity decreased with an increasing order of complexity of the sample matrix (ethanol < standard grain < woody grain < unpeated malt). A similar order was also found in the previous individual phenolic compounds thresholds test, as the phenolic compounds in heavy complex matrices woody grain and unpeated malt were higher than simple ethanol and grain matrices.

In whisky research studies, ethanol solutions are commonly used as the control solutions (ethanol/water 20% (v/v)) for both chemical and aroma attribute threshold measurements (Simpson et al. 1974; Wishart 2006). The ethanol matrix (control) representing the minimum level of aroma interaction giving the lowest recognition threshold for peated malt of about 1% (v/v). The standard grain is the simplest spirit matrix used for whisky blending giving the lowest threshold (about 2% (v/v)) among the whisky matrices (Figure 3.4). Woody grain and unpeated malt are more complicated which yielded much higher peated malt thresholds, particularly for the unpeated malt matrix (about 6% (v/v)), which was about three times higher than that for the standard grain and six times higher than that for the ethanol matrix. This again confirms that different matrices can significantly influence peaty aroma perception

(threshold).

3.1.2.3 Summary

Two approaches of scaling and threshold methods were applied to study the effect of the aroma interaction on peaty aroma perception under different matrix background types. It was concluded that the overall peaty intensity in a blend is not only influenced by the peated malt content, but also strongly affected by other components of the blend. Complicated matrices such as unpeated malt and woody grain could have stronger effect on the perception of peaty aroma, reducing its perceived intensity. These effects must be taken into account during the design of blended whisky, as using the same amount of peated malt in different blends will not necessarily give the same peaty intensity response.

The scaling method has been recognized as a cost-effective sensory assessment. However, it has a major limitation of giving relatively poor quantitative measure. Therefore, the comparability of the test results between different experiments is limited (Lawless and Heymann 1998a; Meilgaard et al. 1999). To a certain extent, this increases the difficulty of the experimental design, since the sample size used in each study is restricted, due to the saturation of the human olfactory receptors.

The scaling test has well-known limitations in its use for sensory response quantification, but with suitable experimental design it can be useful for judging the relative relationships between stimuli of different intensities. In contrast, the threshold method has a benefit of quantifying or providing the measurement of the aroma interaction on the peaty aroma. Unfortunately, this method is much more costly in terms of time and labour.

3.1.3 Determining the nature of aroma interactions (psychological, physicochemical or physiological)

Further work was carried out to investigate the nature of the aroma interactions that effect peaty character. As explained in Chapter 1.5, there are three main types of aroma interactions: physicochemical, physiological and psychological. Since the

sensory panel used in the study was composed of a number of trained and experienced panellists, and the tests were designed to overcome bias, psychological influences were considered to be minimal. Hence, the main objective of this experiment was to identify whether the peaty aroma interactions were caused by physicochemical or physiological effects.

Three blended samples were prepared: standard grain, woody grain and unpeated malt, mixed with 10% (v/v) peated malt. Levels of the eight major phenolic compounds in the headspace of these blends were analysed by SPME analysis (Table 3.8).

Table 3.8 – Mean (three analyses) concentrations of headspace phenolic compounds (mg/L) with % RSD for three whisky blends.

Phenolic compounds	Grain blend	Woody blend	Malt blend
	Mean (%RSD)		
(P1) guaiacol	0.15 (1.2)	0.16 (0.8)	0.14 (4.8)
(P2) 4-methylguaiacol	0.08 (1.0)	0.09 (0.2)	0.08 (3.7)
(P3) *o*-cresol	0.20 (1.2)	0.20 (0.4)	0.18 (5.1)
(P4) phenol	0.26 (1.8)	0.26 (1.2)	0.24 (5.8)
(P5) 4-ethylguaiacol	0.11 (0.9)	0.10 (0.9)	0.09 (4.0)
(P6) *p*-cresol	0.19 (1.1)	0.19 (0.9)	0.17 (4.6)
(P7) *m*-cresol	0.08 (0.5)	0.08 (0.6)	0.08 (2.9)
(P8) 4-ethylphenol	0.12 (0.6)	0.12 (1.2)	0.11 (3.8)
Total phenols	1.19	1.20	1.10

It can be seen in Table 3.8, despite the same amount of peated malt being added to each, most phenolic compounds in this test showed a small difference in headspace between the samples. More, one interesting observation is that the total headspace phenol level is lower in the unpeated malt whisky type than in the standard and the woody grain whiskies. This supports the findings from previous test (Chapter 3.1.1) that malt samples generally contain relatively large quantities of physicochemical active compounds such as long chained esters and fatty acids. These compounds potentially trap the volatile compounds in a liquid phase and inhibit aroma diffusion into the sample headspace (Conner et al. 1994; Piggott et al. 1996; Conner et al. 1999a; Conner et al. 2001; Steele et al. 2004). The preliminary conclusion of this study is that the physiochemical interaction potentially happens when the malt type matrix is involved in blending. However, in this study physicochemical interactions

are considered as minimal factor since in this experiment blends contain a fixed ratio of malt. The question that still remains unresolved is why the standard and woody grain whiskies have different aroma interaction capacities when the headspace levels of the phenols are the same. Some form of physiological interaction provides the only remaining explanation for these observations. This theory is reinforced by reconsidering the previous QDA study of the peated malt (Figure 3.1). Here peaty aroma character is the dominant attribute for this whisky type preventing the perception of other aroma active compounds. It can be concluded that the aroma interactions occurring during blending are complicated physiological interactions. More importantly, the aroma attributes in a whisky blend are not perceived as isolated attributes, but behave as a whole matrix and have an strong influence on each other (Delwiche 2004).

3.1.4 Summary

A series of analytical and sensory experiments were carried out to examine the occurrence of the aroma interaction during blending and the impact of such interactions on the overall aroma character of the final blend. Key findings in this Chapter are concluded as below:

1. Aroma interactions between the components of a blend can alter the perception of peaty character.

2. The composition of a blend (matrix background) can have a significant impact on peaty aroma perception of the final blend.

3. Aroma interactions are primarily caused by physiological effects.

4. The results obtained from PCA analysis of the composition and sensory character of the base whisky matrices (Chapter 3.1.1.6) agree with the observations reported in a previous study of Aylott (2003), that distillation congeners are good markers to distinguish the differences between grain and malt spirits, while cask derived compounds are good indicators of woody character.

3.2 A study of the relationship between peaty character and its related aroma congeners (phenolic compounds)

Phenolic compounds are commonly used in the Scotch whisky industry as an indicator of the peaty character intensity (Aylott 2003). This part of the study was carried out to evaluate the accuracy of this phenol-dependent technique in predicting overall peaty intensity in six commercial peated malts, sourced from different distilleries (in different regions), with different maturation times and conditions (Table 2.5). These samples were selected to cover the general three categories of the peaty whisky in the current whisky market: heavily peated, medium peated and lightly peated (Bathgate and Cook 1989; Dolan 2003).

This study is divided into three parts. Analytical measurements were carried out in the first part to assess the concentration of headspace phenolic compounds, distillate congeners (both major and trace) and maturation derived compounds in the samples. The second part involved the sensory evaluation of the peaty related aroma characters for each sample types. The relationship between the peaty character and the level of phenolic compounds was then assessed to evaluate the accuracy of using the phenol compound levels to predict the overall peaty intensity.

3.2.1 Analytical evaluations

3.2.1.1 Headspace phenolic compounds

Levels of the eight major phenolic compounds in these samples are presented in Table 3.9. It can be seen in Table 3.9 that a difference of the concentration with the phenolic compounds was found between the sample types. The total levels of the phenolic compounds decreasing cross the table. Where the Laphroaig, Lagvulin and Ardbeg are consider as heavily peated, Bunnahabhain and Talisker medium peated and Highland Park light peated.

Table 3.9 – Mean (three analyses) concentrations of phenolic compounds (mg/L) with % RSD for peated malt whiskies.

Phenolic compounds	peated malt Whiskes						p-value
	Laphroaig	Lagavulin	Ardbeg	Bunnahabhain	Talisker	Highland Park	
	Mean (%RSD)						
(P1) guaiacol	1.8 (2.6)	1.3 (25.6)	1.4 (3.8)	0.7 (1.5)	0.4 (0.4)	0.1 (1.1)	P < 0.05
(P2) 4-methylguaiacol	0.7 (1.6)	0.6 (14.8)	0.6 (3.7)	0.3 (0.8)	0.2 (0.5)	0.1 (1.4)	P < 0.05
(P3) *o*-cresol	2.6 (3.1)	2.4 (9.2)	2.1 (3.1)	1.0 (2.3)	0.7 (0.5)	0.2 (1.5)	P < 0.05
(P4) phenol	3.4 (2.1)	4.0 (13.4)	2.7 (4.7)	1.6 (3.0)	1.3 (0.6)	0.2 (3.4)	P < 0.05
(P5) 4-ethylguaiacol	0.8 (1.8)	0.6 (9.5)	0.7 (4.1)	0.3 (1.6)	0.2 (1.3)	0.1 (7.4)	P < 0.05
(P6) *p*-cresol	2.1 (2.3)	2.1 (8.6)	1.6 (4.5)	1.0 (4.2)	0.6 (0.9)	0.1 (2.2)	P < 0.05
(P7) *m*-cresol	0.6 (2.5)	0.6 (8.7)	0.5 (5.4)	0.3 (1.3)	0.2 (2.0)	0.1 (0.8)	P < 0.05
(P8) 4-ethylphenol	1.1 (2.1)	1.1 (6.1)	0.9 (3.5)	0.5 (4.4)	0.3 (1.9)	0.1 (1.1)	P < 0.05
Total Phenols	13.1	12.7	10.5	5.7	3.9	1.0	P < 0.05

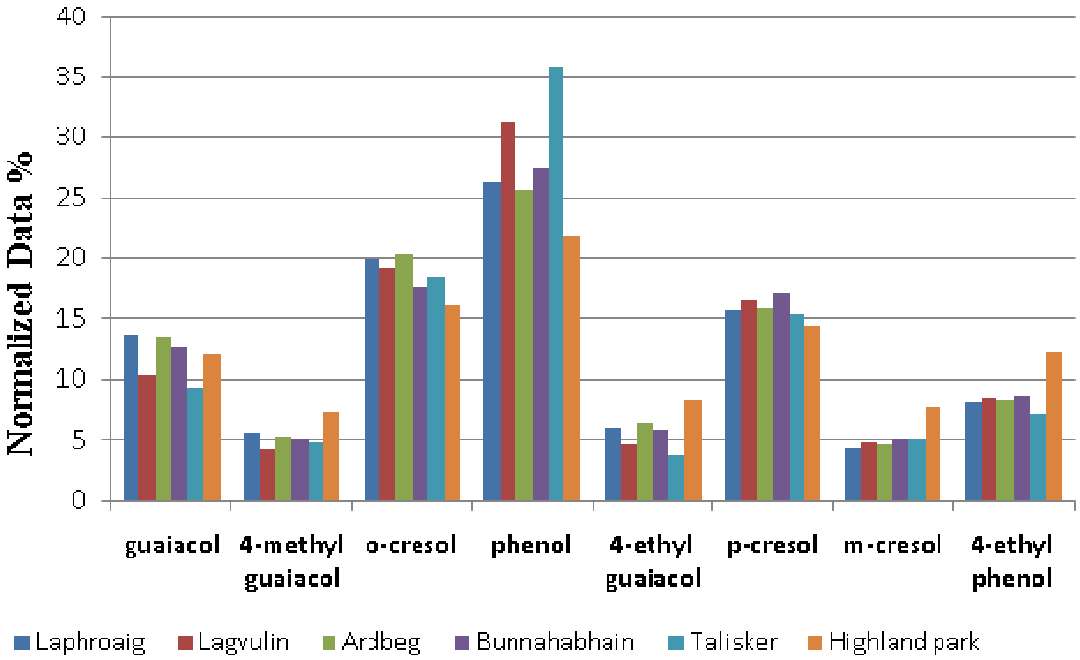
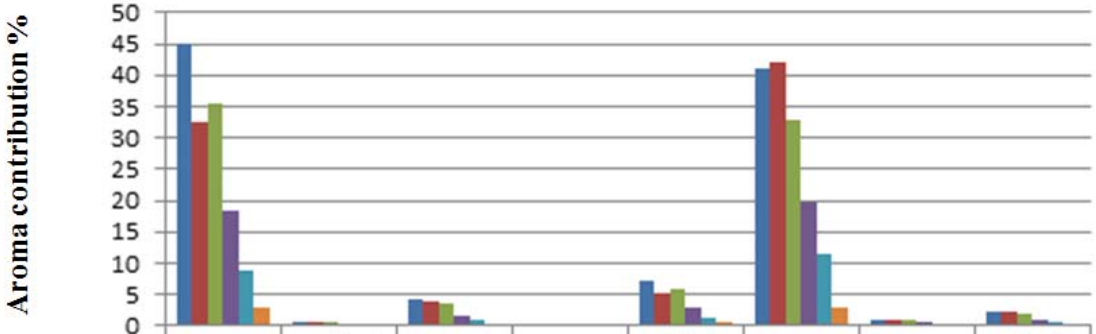


Figure 3.5 – Relative phenol percentage (%) of individual phenolic compounds for peated malt whiskies.

The data from Table 3.9 was normalized by its relative percentage into Figure 3.5. From both Table 3.9 and Figure 3.5 show that the levels of phenol and *o*-cresol are higher than the other phenolic compounds for all samples. These two compounds account for about 50% of the total phenolic compounds. Methyl- and ethyl-guaiacols are found at the lowest levels. Importantly, founding in Figure 3.5 is that the phenols profiles for the various whiskies produced in diverse locations are all remarkably similar. Phenol potential aroma contribution (odour units Chapter 1.5.2.3) was calculated based on the thresholds of the compounds (Table 2.1) and phenol concentration (Table 3.9).



	guaiacol	methyl guaiacol	o-cresol	phenol	ethyl guaiacol	p-cresol	m-cresol	ethyl phenol
Laphroaig	45.08	0.77	4.26	0.18	7.18	41.07	1.01	2.28
Lagavulin	32.61	0.58	3.97	0.21	5.35	41.95	1.06	2.28
Ardbeg	35.35	0.58	3.47	0.14	6.05	32.77	0.84	1.85
Bunnahabhain	18.41	0.32	1.68	0.08	3.03	19.82	0.51	1.07
Talisker	8.70	0.19	1.13	0.07	1.34	11.50	0.33	0.57
Highland Park	2.92	0.07	0.26	0.01	0.74	2.80	0.13	0.25

Figure 3.6 – Phenol potential aroma contribution (odour units)

As shown in Figure 3.6, guaiacols and *p*-cresol have higher aroma contribution according to the odour units (Equation 1 from Chapter 1.5.2.3). These two compounds were therefore likely to be the most important peat-related compounds giving the peaty character.

3.2.1.2 Distillate congeners

Both the major and the trace distillate congeners were assessed by GC and the results are shown in Table 3.10Table 3.11. The results showed statistically significant evidence of the differences in the distillate congeners between different peated malt sample types ($p < 0.05$). The levels of the distillate congeners showed relatively high concentrations in all sample types compared to the grain samples (Chapter 3.1.1).

Table 3.10 – Mean (three analyses) concentrations (40% v/v) of major (g/100L) distillate congeners with % RSD for commercial peated malt whiskies and ANOVA, using whisky types as a factor.

Major Congeners	Laphroaig	Lagavulin	Ardbeg	Bunnahabhain	Talisker	Highland Park	p-value
	Mean (%RSD)						
(D1) acetaldehyde	7.5 (5.9)	10.7 (3.2)	7.8 (13.4)	8.7 (11.5)	11.8 (3.7)	9.0 (9.6)	$p < 0.05$
(D2) ethyl acetate	33.6 (6.5)	48.2 (6.4)	44.3 (2.3)	32.9 (5.2)	52.6 (5.8)	49.4 (7.0)	$p < 0.05$
(D3) acetal	7.7 (7.7)	8.5 (7.6)	6.8 (6.4)	8.2 (9.6)	10.9 (5.9)	6.2 (10.9)	$p < 0.05$
(D4) methanol	4.8 (4.9)	5.8 (4.8)	5.1 (2.0)	5.1 (3.8)	5.5 (4.0)	6.8 (4.9)	$p < 0.05$
(D5) n-propanol	46.5 (2.8)	43.4 (2.9)	44.9 (1.4)	42.4 (2.2)	45.3 (3.1)	40.5 (2.7)	$p < 0.05$
(D6) iso-butanol	70.6 (1.8)	67.2 (1.4)	102.8 (3.1)	95.3 (1.5)	85.6 (1.4)	68.5 (0.9)	$p < 0.05$
(D7) iso-amyl acetate	2.5 (11.6)	2.4 (12.0)	3.7 (18.2)	3.6 (14.5)	2.2 (17.5)	2.0 (13.2)	$p < 0.05$
(D8) n-butanol	2.1 (7.3)	1.8 (7.4)	1.7 (4.8)	1.6 (5.2)	1.5 (5.7)	1.7 (6.1)	$p < 0.05$
(D9) 2-methyl-1-butanol	52.7 (2.0)	45.7 (1.8)	65.8 (2.4)	63.3 (1.5)	54.1 (1.7)	49.7 (1.1)	$p < 0.05$
(D10) 3-Methyl-1-butanol	138.2 (1.9)	134.2 (1.2)	157.9 (2.5)	151.6 (1.5)	151.8 (1.8)	134.4 (1.3)	$p < 0.05$
Total major congeners	366.2	367.9	440.8	412.7	421.3	368.2	$p < 0.05$

Table 3.11 – Mean (three analyses) concentrations (40% v/v) of trace (mg/L) distillate congeners with % RSD for commercial peated malt whiskies and ANOVA, using whisky types as a factor.

Trace Congeners	Laphroaig	Lagavulin	Ardbeg	Bunnahabhain	Talisker	Highland Park	p-value
	Mean (%RSD)						
(D11) ethyl hexanoate	1.9 (3.1)	3.9 (3.2)	2.1 (4.4)	2.0 (6.5)	3.9 (3.7)	2.7 (3.3)	$p < 0.05$
(D12) ethyl octanoate	15.3 (0.3)	26.6 (1.2)	16.7 (1.8)	17.8 (1.6)	32.3 (2.6)	18.2 (1.1)	$p < 0.05$
(D13) ethyl decanoate	26.8 (1.5)	43.0 (0.3)	35.3 (2.2)	43.0 (1.2)	68.5 (2.2)	21.0 (2.6)	$p < 0.05$
(D14) ethyl dodecanoate	18.4 (0.3)	19.2 (0.7)	23.5 (1.8)	32.3 (1.1)	35.8 (1.6)	7.3 (1.2)	$p < 0.05$
(D15) ethyl-tetradecanoate	3.4 (2.8)	1.4 (1.4)	4.6 (1.5)	6.8 (1.2)	2.9 (1.2)	0.5 (6.7)	$p < 0.05$
(D16) ethyl hexadecanoate	11.0 (0.3)	1.3 (0.9)	11.2 (0.5)	13.2 (0.5)	3.1 (0.7)	0.6 (1.4)	$p < 0.05$
(D17) ethyl l9-hexadecenoate	8.8 (0.7)	2.1 (1.4)	9.1 (1.9)	13.8 (1.3)	5.7 (0.2)	0.9 (0.5)	$p < 0.05$
(D18) 2-phenethyl acetate	5.4 (3.3)	3.2 (6.5)	5.5 (2.5)	6.6 (6.0)	2.8 (5.7)	2.0 (2.7)	$p < 0.05$
(D19) 2-phenethyl alcohol	36.6 (0.9)	27.1 (0.9)	29.6 (1.9)	32.3 (2.6)	29.1 (1.2)	24.2 (1.9)	$p < 0.05$
Total trace congeners	127.6	127.8	137.6	167.8	184.1	77.4	$p < 0.05$

3.2.1.3 Maturation-derived compounds

Maturation-derived compounds were measured for the six commercial peated malt whisky samples (Table 3.12). Results showed significant differences in all maturation-derived compounds between the six peated malt samples ($p < 0.05$). It was also noted that the total maturation-derived compounds did not tend to increase with the age of the whisky. For example, Highland Park had higher levels of these congeners than the other products, but it was not the oldest whisky in this study.

Table 3.12 – Mean (three analyses) concentrations of maturation-derived compounds (mg/L) with % RSD for peated malt whiskies and p-value from ANOVA, using whisky types as a factor

Maturation-derived compounds (mg/L)							
Analyte	**Laphroaig (7 years)**	**Bunnahabhain (9 years)**	**Ardbeg (10 years)**	**Highland Park (12 years)**	**Lagavulin (16 years)**	**Talisker (18 years)**	p-value
	Mean (%RSD)						
(W1) gallic acid	12.4 (0.4)	13.6 (0.7)	5.6 (1.4)	19.6 (3.9)	5.0 (3.5)	6.8 (2.2)	$p < 0.05$
(W2) ellagic acid	16.7 (0.1)	21.3 (0.1)	13.3 (0.2)	30.0 (0.3)	13.6 (0.1)	15.3 (0.4)	$p < 0.05$
(W3) coniferaldehyde	0.7 (1.8)	0.5 (4.7)	1.0 (0.9)	1.5 (1.4)	0.6 (5.7)	0.6 (5.9)	$p < 0.05$
(W4) vanillin	2.7 (1.5)	3.5 (1.3)	3.9 (0.8)	4.3 (0.8)	2.4 (0.3)	2.6 (0.6)	$p < 0.05$
(W5) vanillic acid	1.1 (2.8)	1.4 (2.7)	1.8 (0.7)	2.4 (0.3)	1.6 (1.5)	2.0 (1.3)	$p < 0.05$
(W6) sinapaldehyde	1.2 (10.4)	0.6 (12.0)	1.1 (3.6)	1.6 (0.1)	0.7 (2.1)	0.5 (7.0)	$p < 0.05$
(W7) syringaldehyde	4.8 (1.2)	6.4 (4.3)	7.3 (1.0)	8.4 (2.2)	5.0 (3.3)	4.6 (4.0)	$p < 0.05$
(W8) syringic acid	2.2 (3.9)	3.2 (2.2)	3.4 (2.0)	5.2 (2.9)	3.2 (11.5)	3.3 (4.7)	$p < 0.05$
(W9) scopoletin	0.3 (9.3)	0.2 (23.6)	1.2 (5.0)	0.9 (7.2)	0.7 (3.9)	0.9 (7.3)	$p < 0.05$
(W10) 5-HMF	1.4 (1.4)	1.4 (0.9)	1.0 (1.4)	5.3 (0.2)	13.1 (0.3)	7.8 (0.5)	$p < 0.05$
Total	43.5	52.1	39.6	79.2	45.9	44.4	$p < 0.05$

3.2.2 Peaty whiskies study

Sensory assessment was carried out on the samples to evaluate the peat related aroma attributes, including medicinal, smoky, burnt and overall peaty intensity. Statistical analysis was also applied to evaluate the correlation between the aroma characters and the overall peaty intensity. QDA scores and the related aroma profile are shown in Table 3.13 and Figure 3.6, respectively.

Table 3.13 – QDA scores of aroma attributes for commercial peated malt whiskies and ANOVA, using whisky types as a factor. Data on the scale of 0 – 3.

Commercial peated malt	Overall peaty intensity	Burnt	Smoky	Medicinal
Laphroaig	2.8	1.9	1.9	1.5
Lagavulin	2.0	1.4	1.5	1.3
Ardbeg	1.6	1.1	1.1	1.4
Bunnahabhain	1.5	1.2	1.2	0.9
Talisker	1.0	0.5	0.5	0.9
Highland Park	0.8	0.7	0.6	0.7
p-value	0.0001*	0.0001*	0.0001*	0.0017*

**p-values < 0.05 showing significant differences of aroma attributes between the commercial peated malt whiskies*

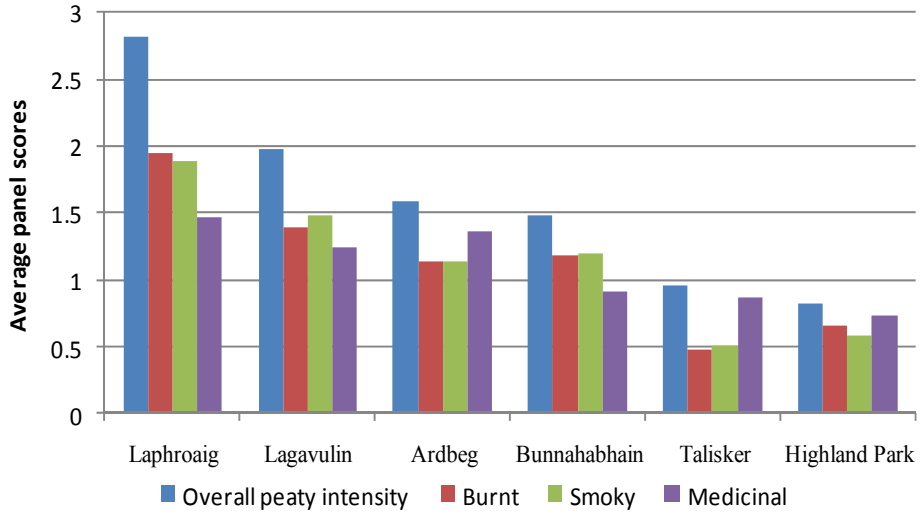


Figure 3.7 – Aroma profiles of peated malt whiskies (same data as Table 3.13), Data on the scale of 0 – 3.

Table 3.13 shows statistically highly significant ($P < 0.05$) evidence of differences in all (overall peaty, burnt, smoky and medicinal) the peat related aroma attributes between the samples. Figure 3.6 shows a trend of decline in the peaty related aroma attributes across the samples taken from different regions, Islay (Laphroaig, Lagavulin, Ardbeg

and Bunnahabhain) and the Islands (Talisker and Highland Park). In addition, by comparing the burnt, smoky and medicinal with Overall peaty intensity, it was found that, burnt (R = 0.97) and smoky (R = 0.97) characters are well correlated with the overall peaty intensity, followed by medicinal (R = 0.87). Similar results were also found in the earlier study of aroma interaction on peaty aroma by using the scaling method (Chapter 3.1.2.1). It was suggested that the overall peaty intensity of peated malt samples is highly correlated to smoky and burnt characters, whereas the medicinal attribute is less contributed.

3.2.3 Exploration of relationships between peaty character and phenolic compounds

It is generally accepted by the whisky industry that the intensity of peaty character in whisky is positively correlated with the levels of phenolic compounds present. This Chapter was carried out to investigate the effect of aroma interaction on human perception in relation to the peaty character.

3.2.3.1 Principal Component Analysis

Thirty seven compounds representing distillate congeners (D), maturation-derived compounds (W) and the peaty related compounds (P) were evaluated by PCA, against the four peat related aroma characters (overall peaty intensity, burnt, smoky and medicinal). Results are shown in Figure 3.8 and Figure 3.9.

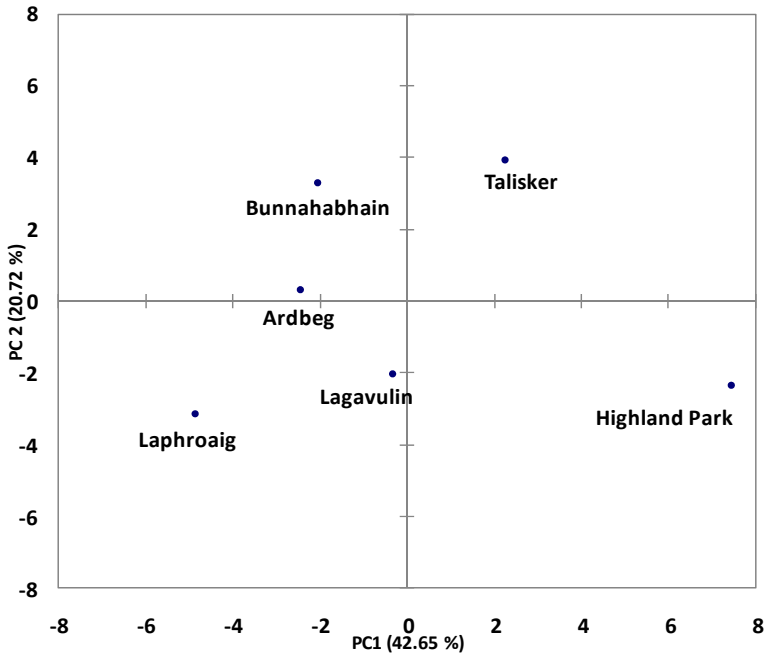


Figure 3.8 – Scores plot for the peated malt sample principal component (62.97% variance explained by two PCs).

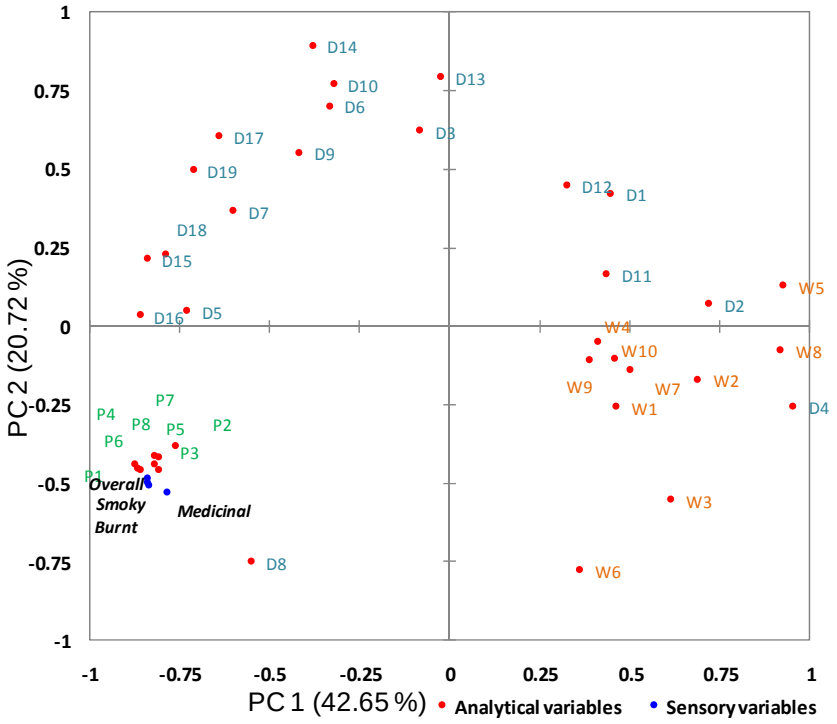


Figure 3.9 – Loadings plot for the principal components of analytical and sensory attributes corresponding to chemical compounds and aroma characters, respectively. Loading labels were given in Table 3.10 to Table 3.11. D (Distillate character congeners), P (Peaty character congeners), W (Woody character congener).

Figure 3.8 and Figure 3.9 give an overview of the relationship of the peated malt congeners and their characterized distillate characters i.e. chemical compounds and aroma attributes. It can be seen in Figure 3.8 that the samples with higher levels of phenolic compounds are likely to exhibit higher level of overall peaty intensity. Laphroaig, Lagavulin, Ardbeg and Bunnahabhain, located on the left side of the plot with negative PC2, are characterised by high levels of phenolic compounds, and these four peated malt whiskies were samples from Islay. Talisker and Highland Park, located on the right side of the plot with positive PC2, were characterised by relatively low levels of phenols.

As expected, phenolic compounds shown as a cluster and related to all peaty related aroma attributes. It was found that other aromatic compounds, including distillate congeners, are dispersed in the plot. Bunnahabhain is particularly rich in distillate congeners and maturation-derived compounds are particularly abundant in Highland Park (Table 3.11). Distillate congeners and maturation-derived compounds do not appear to have a clear relationship with peaty related aroma, due to all the samples were peated malt with relatively common distillate and maturation character (Table 3.2Table

3.3).

3.2.3.2 Linear Regression Analysis

Linear regression analysis was used to evaluate the relationship between individual phenolic compounds and peaty related aroma attributes. Correlation coefficients were calculated to determine the association between the analytical and sensory variables. The resulting R^2 values are shown in Table 3.14 and an example of the relationship between total phenols and peaty intensity is illustrated Figure 3.10.

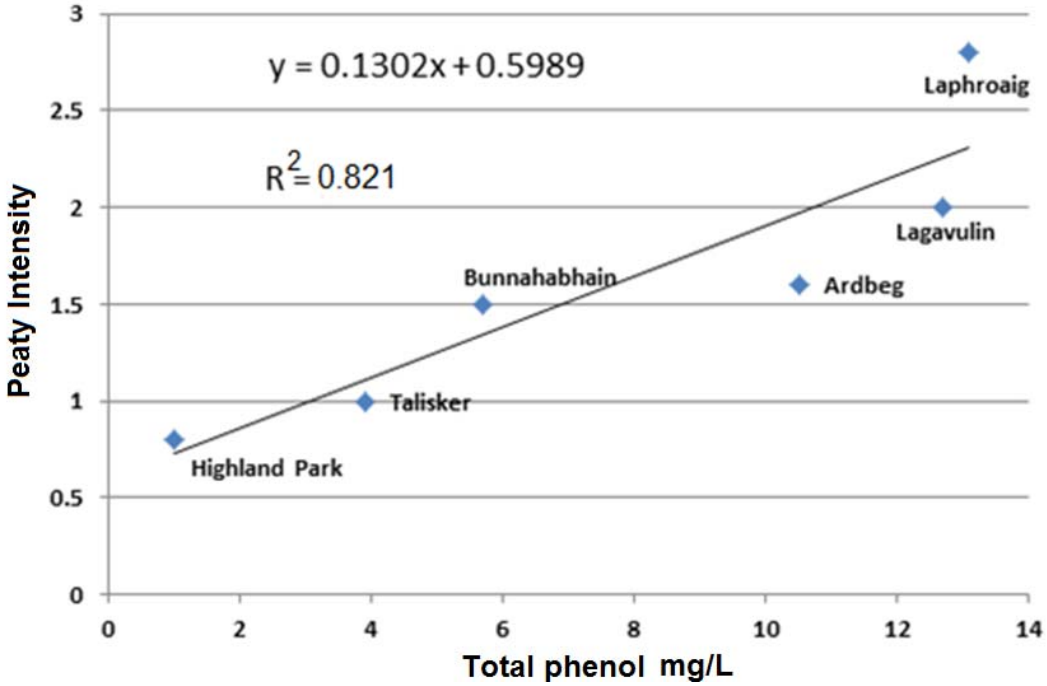


Figure 3.10 – Relationship between total phenol and peaty intensity.

Table 3.14 – Output of linear regression analysis (R squared) of phenolic compounds and peaty related attributes.

Analyte	Overall peaty intensity	Burnt	Smoky	Medicinal
(P1) guaiacol	0.870*	0.841*	0.850*	0.960*
(P2) 4-methylguaiacol	0.889*	0.859*	0.859*	0.951*
(P3) *o*-cresol	0.810*	0.769*	0.799*	0.920*
(P4) phenol	0.729*	0.681*	0.740*	0.750*
(P5) 4-ethylguaiacol	0.830*	0.821*	0.821*	0.980*
(P6) *p*-cresol	0.799*	0.780*	0.830*	0.850*
(P7) *m*-cresol	0.780*	0.750*	0.799*	0.859*
(P8) 4-ethylphenol	0.810*	0.790*	0.841*	0.889*
Total phenol	0.821*	0.780*	0.821*	0.889*

**p-value <0.05 showing significant correlations between the phenolic compounds and the*

In Table 3.14, as expected all the phenolic compounds show significant correlations with the peaty related aroma attributes. The overall values of R^2 are high, particularly for medicinal. In a previous aroma interaction study (Chapter 3.1.3), it was found that using the traditional phenols analysis itself was not accurate enough for predicting the peaty intensity in the blended samples (Table 3.8). The results obtained from this experiment confirmed that the total phenols measurement is a still useful preferred analytical measure of peaty character, when the matrices background between test sample all same (physiological interaction influence free).

3.2.4 Summary

Total phenols have traditionally been used by the whisky industry for an indication of the peaty character. It was demonstrated in this study that the phenolic compounds were able to predict the peaty intensity where all whiskies had similar matrix backgrounds (i.e. were all malt whiskies). However, it has been previously proved in Chapter 3.1.3, that although a blend contained the same concentration of phenolic compounds, the overall peaty perception may be influenced significantly by the background matrix of the blend. Therefore, based on all previous obtained data and phenomena, it could be concluded that the traditional prediction of peaty intensity based on the level of phenolic compounds is suitable for same backgrounds, but when the matrix backgrounds differ the phenol method cannot adopt with the different background circumstances.

In addition, the similarity in the relative abundance of phenolic compounds and the similar correlations with burnt, smoky and medicinal, this might suggest that the most important factor for peated malts used in blending is the level of peating rather than which distillery they are from. And the amount of peat level used in a blend is relatively easy to be measured by phenol test instrument.

3.3 Aroma interaction capacity study for Grain and Malt whiskies

Multiple samples of the three basic whisky types (standard grain, unpeated malt and woody grain) were subjected to various analytical and sensory evaluations to determine differences within each category. Further scaling and threshold tests were also carried out to compare Aroma Interaction Capacities between similar styles of whiskies.

3.3.1 A comparative study of standard grain whiskies

In previous study Chapter 3.1, the differences of grain and malt were general studied for its chemical, sensory and Aroma interaction capacity (AIC). It was found that the standard grain is a relatively simpler matrix compare with malt whisky. To further explore the linkage of grain whiskies chemical, sensory and aroma interaction capacity, a comparative study of four grain whiskies was carried out. Four grain whiskies were selected to cover the most used grain whisky in industry. Sample details can be found in Table 2.5.

3.3.1.1 Chemical profile comparison of the standard grain whiskies

Chemical profiles of the standard grain whiskies are shown in Table 3.15. Only the distillate congeners were examined as the phenol and woody related congener levels were very low and not significantly different between samples.

Table 3.15 – Mean (three analyses) concentrations (40% abv v/v) of major (g/100L) and trace (mg/L) distillate congeners for standard grain whiskies with % RSD and ANOVA, using whisky types as a factor.

Volatile compounds	Invergordon	Girvan	Port Dundas	Cameron Bridge	p-value
	Major Congeners / mean (%RSD) g/100 L				
(D1) acetaldehyde	1.8 (6.3)	2.3 (4.9)	1.5 (3.9)	2.4 (2.4)	$P < 0.05$
(D2) ethyl acetate	20.4 (2.0)	17.4 (0.9)	13.5 (1.1)	17.2 (1.0)	$P < 0.05$
(D3) acetal	3.2 (3.1)	3.5 (1.7)	0.9 (6.2)	1.4 (4.0)	$P < 0.05$
(D4) methanol	6.4 (2.4)	5.3 (1.1)	9.6 (1.0)	10.9 (1.1)	$P < 0.05$
(D5) n-propanol	121.6 (1.8)	62.3 (0.3)	68.9 (0.8)	57.9 (0.1)	$P < 0.05$
(D6) iso-butanol	41.7 (1.7)	60.9 (0.6)	32.0 (1.0)	60.5 (0.2)	$P < 0.05$
(D7) iso-amyl acetate	1.3 (7.7)	1.8 (5.6)	0.9 (17.6)	1.2 (16.9)	$P < 0.05$
(D8) n-butanol	ND	ND	ND	ND	
(D9) 2-methyl-1-butanol	1.9 (3.1)	0.8 (0.1)	0.7 (7.9)	2.4 (0.1)	$P < 0.05$
(D10) 3-methyl-1-butanol	3.6 (4.3)	1.7 (0.1)	1.5 (0.1)	5.6(1.0)	$P < 0.05$
Total major congeners	201.9	156.1	129.5	159.5	
(D11) ethyl hexanoate	ND	ND	ND	ND	
(D12) ethyl octanoate	0.3 (6.2)	0.4 (2.4)	0.5 (2.9)	1.0 (4.1)	$P < 0.05$ $P < 0.05$
(D13) ethyl decanoate	0.6 (1.6)	1.0 (3.1)	1.2 (2.7)	2.4 (3.7)	$P < 0.05$

(D14) ethyl dodecanoate	0.4 (3.4)	0.8 (0.3)	0.8 (2.5)	1.3 (11.3)	$P < 0.05$
(D15) ethyl tetradecanoate	0.0	0.2 (1.5)	0.2 (2.1)	0.2 (5.2)	$P < 0.05$
(D16) ethyl hexadecanoate	0.3 (1.4)	0.3 (4.8)	0.4 (3.6)	0.6 (3.0)	$P < 0.05$
(D17) ethyl 9-hexadecenoate	0.0	0.0 (24.2)	0.0 (60.2)	0.2 (15.2)	$P < 0.05$
(D18) 2-phenethyl acetate	ND	ND	ND	ND	
(D19) 2-phenethyl alcohol	1.2 (1.0)	0.8 (1.0)	1.0 (1.1)	1.9 (2.6)	$P < 0.05$
Total trace congeners	2.8	3.5	4.1	7.6	$P < 0.05$

ANOVA showed significant differences in the congener contents of distillate compounds between the samples ($p < 0.05$). It was observed that for all standard grain samples, both major and trace distillate congeners were relatively low or even absent. The main reason for this is because the volatile compounds were largely removed during the continuous distillation. No correlation between total major and trace levels was observed. Also it was noticed, that the variations between each congener was large, and the total chemical content between different distilleries varied greatly as well.

3.3.1.2 Sensory comparison of the standard grain whiskies

In this section, aroma profiles of all samples were assessed by QDA. Eight basic aroma attributes were selected, but peaty and woody characters were not considered as these samples were produced without peating process and were only maturated in refill casks (minimum maturation time of 3 years to remove the immaturity). QDA outputs together with the statistical output are shown in Table 3.16.

Table 3.16 – QDA scores of aroma attributes for each standard grain whisky and ANOVA, using whisky types as a factor (at 5% significance level).

Aroma Attribute	Invergordon	Girvan	Port Dundas	Cameron Bridge	p-value
Sweet/Floral	1.0	0.3	0.6	0.8	0.001*
Fruity/Solventy	0.8	0.4	0.5	0.8	0.001*
Green/Grassy	0.4	0.3	0.6	0.4	0.191
Sour	0.6	0.7	0.4	0.4	0.256
Cereal/Nutty	0.5	0.4	0.2	0.4	0.113
Feinty	0.5	0.7	0.3	0.4	0.006*
Sulphury	0.4	1.4	0.2	0.2	0.001*
Soapy/Oily	0.6	0.5	0.6	0.4	0.441

**p < 0.05 showing the significant differences of relevant aroma attributes between the four standard grain whiskies*

It can be seen in Table 3.16 that all the samples exhibited different aroma profiles. The levels of sweet/floral, fruity/solventy, feinty and sulphury are significantly different

between the whisky samples ($p < 0.05$). For clearer comparison, QDA scores were also presented in a radar plot Figure 3.11.

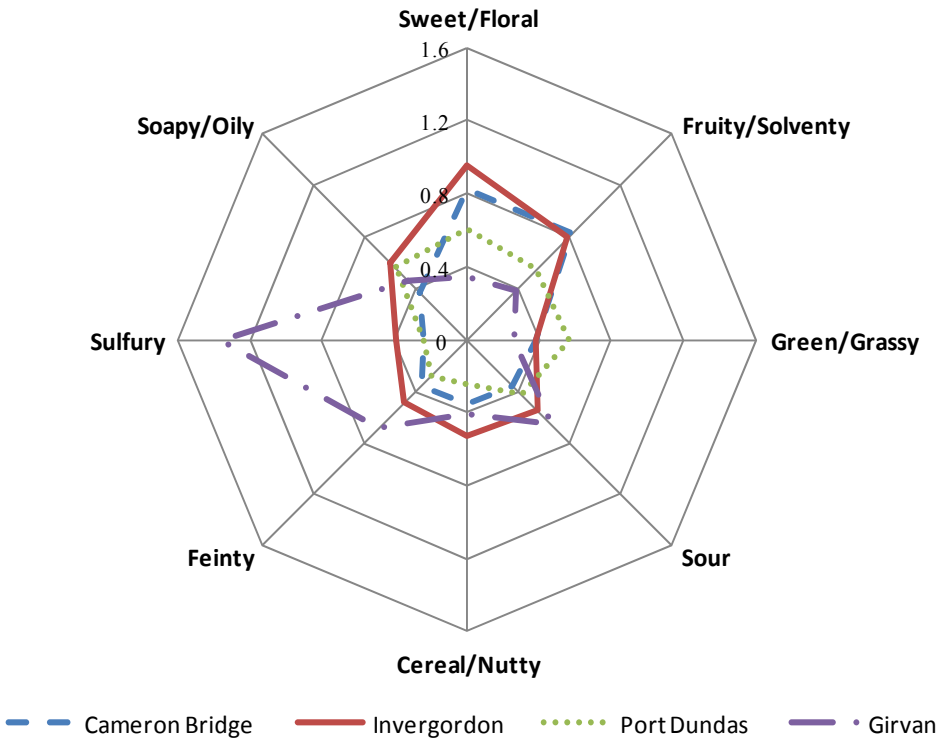


Figure 3.11 – Radar plot of aroma profiles for four standard grain whiskies.

The standard grain whiskies are normally considered as light-bodied spirits, since most of the volatile aroma active compounds have been removed during the continuous distillation process. It is quite unusual for a grain whisky to have similar behaviour to Girvan, which has a relatively strong and heavy sulphury aroma. It was believed that this particular spirit has not yet reached the minimum requirement of maturation to remove the immature characters or possibly has been contaminated by the cask carry-over from a previous fill, or lack of copper during it been distilled. Also the sulphury notes are potentially suppressing other sensory attributes of the spirit, based on the spider plot.

3.3.1.3 Aroma Interaction Capacities of the standard grains

The aroma interaction capacities of each of the four standard grain whiskies were measured using both a scaling test and by threshold measurement.

AIC by scaling

Similar to the previous Chapters 3.1.1, blended samples were prepared using the same

amount of the peated malt (10% (v/v) in the four standard grain whiskies. To simplify the experimental design (explained in Chapter 3.1.2.2), only the overall peaty intensity was studied. Statistical analysis was then applied to determine the difference of the overall peaty intensity between the samples. Results are shown in Figure 3.12.

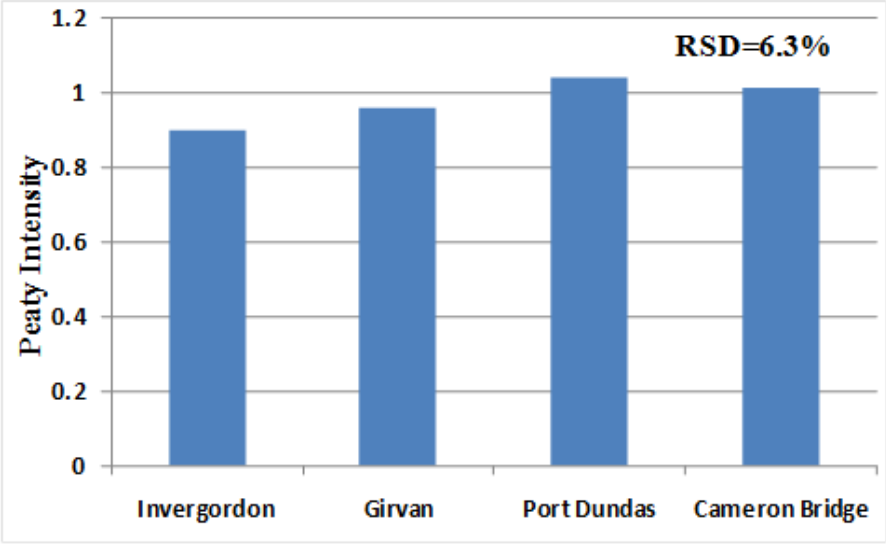


Figure 3.12 – Overall peaty intensity for standard grain based blends.

ANOVA showed no significant (p-value = 0.7981) difference in the overall peaty intensity and a relatively small variation between the samples (RSD = 6.3%). These results indicate that different standard grain whiskies all have similar aroma interaction capacities. The sulphury character of the Girvan spirit did not seem to significantly affect the intensity of the added peaty character.

AIC by threshold measurement

Threshold of peated malt in the four standard grain whiskies are shown in Figure 3.13.

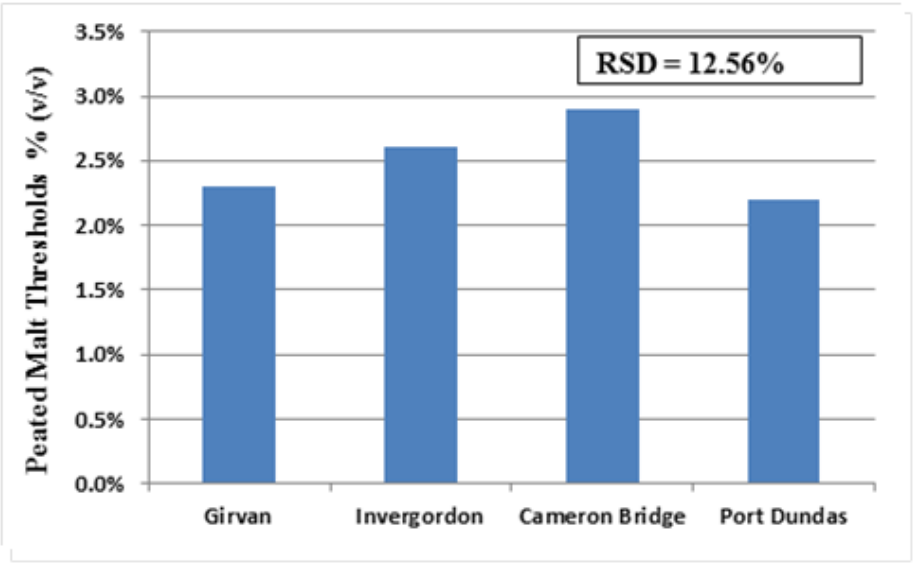


Figure 3.13 – Threshold levels of peated malt in standard grain whisky matrices.

There were no significant (p-value = 0.325) differences in the peated malt thresholds between the standard grain whiskies and only a small variation of the threshold levels (RSD = 12.56%) was found between the samples. It was also observed that these four whisky matrices gave relatively low peated malt threshold values (an average of 2.5% (v/v)). The thresholds for these four grain whiskies are not significantly different from the threshold of the standard grain used in the previous experiments (ie for the vatted grain matrix 2% (v/v) reported in Figure 3.13). Threshold measurements have shown that the standard grain whiskies obtained from different distilleries had similar aroma interaction capacities, affecting the perception of peaty character in the blends to a similar degree.

3.3.2 A comparative study of unpeated malt whiskies

In previous study Chapter 3.1, the differences of grain and malt were general studied for its chemical, sensory and Aroma interaction capacity (AIC). It was found that the unpeated malt whisky has more complex chemical and aroma profiles (Table 3.1Table 3.5). Meantime malt also demonstrated higher AIC compare with standard grain whisky (Figure 3.4). To further explore the linkage of malt whiskies chemical, sensory and aroma interaction capacity, a comparative study of eight unpeated malt whiskies was carried out. Eight malt whiskies were selected from Scotch industry samples by it aroma categories to cover the general aroma character of Scotch malt whisky. Sample details can be found in Table 2.5.

3.3.2.1 Chemical profile comparison of the unpeated malt whiskies

Table 3.17 – Mean (three analysis) concentrations of major (g/100L) and trace (mg/mL) distillate congeners (sample strength) for eight unpeated malt samples with % RSD and ANOVA, using whisky types as a factor.

Volatile compounds	Glendullan	Clynelish	Benrinnes	Blair Athol	Cardhu	Dailuaine	Linkwood	Knockando	
	Major Congeners / mean (%RSD)								
(D1) acetaldehyde	4.2 (11.5)	5.7 (11.2)	4.2 (13.9)	8.5 (15.3)	6.4 (17.1)	6.5 (2.1)	8.6 (5.2)	7.0 (14.7)	$p < 0.05$
(D2) ethyl acetate	43.5 (13.7)	32.9 (17.9)	35.8 (12.1)	35.6 (6.6)	48.7 (7.2)	32.0 (41.4)	39.2 (5.4)	23.5 (7.3)	$p < 0.05$
(D3) acetal	3.5 (38.7)	4.9 (44.6)	3.2 (34.5)	7.1 (36.2)	5.2 (29.8)	5.4 (61.5)	6.5 (33.1)	5.2 (36.6)	$p < 0.05$
(D4) methanol	4.4 (9.0)	4.8 (4.7)	4.9 (7.5)	5.6 (5.1)	5.4 (4.9)	5.3 (6.5)	3.9 (2.2)	4.3 (5.4)	$p < 0.05$
(D5) n-propanol	53.7 (6.9)	49.0 (2.7)	48.3 (4.0)	60.7 (3.5)	50.5 (3.2)	44.7 (3.7)	46.4 (3.2)	54.3 (4.1)	$p < 0.05$
(D6) iso-butanol	59.1 (6.2)	63.3 (2.0)	62.6 (3.2)	68.7 (2.6)	80.7 (2.4)	72.4 (3.1)	58.5 (2.2)	99.0 (3.0)	$p < 0.05$
(D7) iso-amyl acetate	3.9 (9.5)	3.0 (1.8)	4.2 (5.6)	2.9 (2.7)	5.3 (2.6)	4.4 (13.5)	4.0 (5.6)	2.7 (3.1)	$p < 0.05$
(D8) n-butanol	1.1 (24.4)	1.2 (11.2)	1.6 (12.2)	1.4 (8.0)	1.2 (6.9)	1.2 (6.9)	0.9 (9.5)	1.5 (10.9)	$p < 0.05$
(D9) 2-methyl-1-butanol	41.1 (6.9)	39.2 (2.7)	47.0 (4.1)	46.2 (3.6)	53.2 (3.3)	51.7 (3.4)	40.7 (3.3)	69.2 (3.8)	$p < 0.05$
(D10) 3-Methyl-1-butanol	154.7 (6.2)	120.0 (1.9)	132.9 (3.3)	146.5 (3.4)	160.4 (3.1)	157.9 (3.1)	121.9 (2.5)	155.8 (3.6)	$p < 0.05$
Total major congeners	369.2	324.0	344.7	383.2	417.0	381.5	330.6	422.5	$p < 0.05$
	Trace Congeners / mean (%RSD)								
(D11) ethyl hexanoate	1.6 (1.8)	1.9 (0.9)	1.4 (0.9)	1.2 (1.2)	1.5 (0.8)	1.2 (3.0)	1.4 (4.6)	2.2 (1.0)	$p < 0.05$
(D12) ethyl octanoate	11.7 (0.6)	14.1 (0.5)	10.3 (0.2)	9.8 (0.5)	11.6 (1.0)	10.0 (1.3)	6.8 (4.1)	13.8 (1.3)	$p < 0.05$
(D13) ethyl decanoate	24.3 (0.7)	35.5 (0.3)	23.9 (0.1)	21.6 (1.0)	22.0 (1.1)	21.8 (1.5)	14.9 (1.4)	26.4 (0.8)	$p < 0.05$
(D14) ethyl dodecanoate	10.1 (1.6)	19.5 (0.5)	12.2 (0.8)	10.8 (0.9)	9.7 (1.0)	10.8 (1.9)	4.1 (1.3)	13.0 (0.7)	$p < 0.05$
(D15) ethyl-tetradecanoate	2.5 (1.4)	7.8 (0.8)	3.2 (0.2)	1.7 (0.4)	1.8 (1.2)	2.3 (1.8)	0.6 (1.5)	3.6 (1.6)	$p < 0.05$
(D16) ethyl hexadecanoate	12.7 (0.8)	12.0 (0.4)	11.0 (0.2)	8.0 (0.3)	5.6 (1.2)	10.3 (0.5)	2.7 (0.3)	12.4 (0.2)	$p < 0.05$
(D17) ethyl 9-hexadecenoate	10.0 (1.5)	7.7 (1.6)	9.5 (0.6)	5.9 (1.0)	5.2 (1.0)	8.5 (1.4)	1.5 (6.0)	7.4 (1.8)	$p < 0.05$
(D18) 2-phenethyl acetate	3.1 (0.9)	2.8 (0.5)	6.5 (0.6)	3.4 (1.6)	5.7 (0.9)	6.4 (2.6)	3.7 (2.6)	4.6 (1.7)	$p < 0.05$
(D19) 2-phenethyl alcohol	32.0 (1.7)	24.9 (2.2)	12.6 (0.8)	30.0 (1.0)	22.1 (1.8)	29.9 (2.3)	22.9 (4.1)	29.6 (1.1)	$p < 0.05$
Total trace congeners	108.0	126.2	90.6	92.4	85.2	101.2	58.6	113.0	$p < 0.05$

Statistically significant differences were found between the eight whiskies in terms of the entire major and trace distillate congeners ($p < 0.05$). In comparison to the standard grain whiskies, the levels of the distillate compounds in unpeated malts are much higher. It was also found that the total major (Min = 324, Max = 423) and trace (Min = 59, Max = 126) congeners between the eight unpeated malt samples varied greatly. Although yeast produces qualitatively much the same aroma compounds, the quantitative composition can vary greatly between distilleries. These compounds formed in the fermentations are greatly affected by the fermentation and distillation conditions which have been applied (Suomalainen and Lehtonen 1976; Lehtonen 1983b).

3.3.2.2 Sensory comparison of the unpeated malt whiskies

The same eight basic aroma attributes (Chapter 3.3.1.2) were used to profile the eight unpeated malt whiskies. In order to obtain supportive data, a high number of scores (64) is required in this test, might result in sensory saturation of the panellists during the test. To overcome these samples were assessed in two separate experiments, Test 1 and Test 2. This method had disadvantages when cross comparing the samples, but avoiding assessor fatigue was considered to be the preferred option here. Results for the two were combined and compared together in further statistical analysis.

QDA of unpeated malt – Test 1

Table 3.18 – QDA scores of aroma attributes for unpeated malt whiskies and ANOVA, using whisky types as a factor.

Aroma Attribute	Linkwood	Clynelish	Knockando	Dailuaine	p-value
Sweet/Floral	1.3	1.5	1.0	1.2	0.221
Fruity/solventy	1.3	2.0	0.9	1.3	0.001*
Green/Grassy	1.1	1.0	0.5	1.1	0.029*
Sour	0.8	0.5	0.8	0.6	0.333
Cereal/Nutty	0.7	0.6	1.1	0.8	0.007*
Feinty	0.9	0.7	1.4	1.0	0.002*
Sulphury	0.6	0.5	1.3	0.7	0.003*
Soapy/Oily	0.9	1.0	1.5	0.8	0.002*

**p-values < 0.05 showing the significant differences of the aroma attributes between the four unpeated malt whiskies*

In the first test aroma profiles were obtained for Linkwood, Clynelish, Knockando and Dailuaine. The results obtained from QDA and ANOVA are shown in Table 3.18 and Figure 3.14.

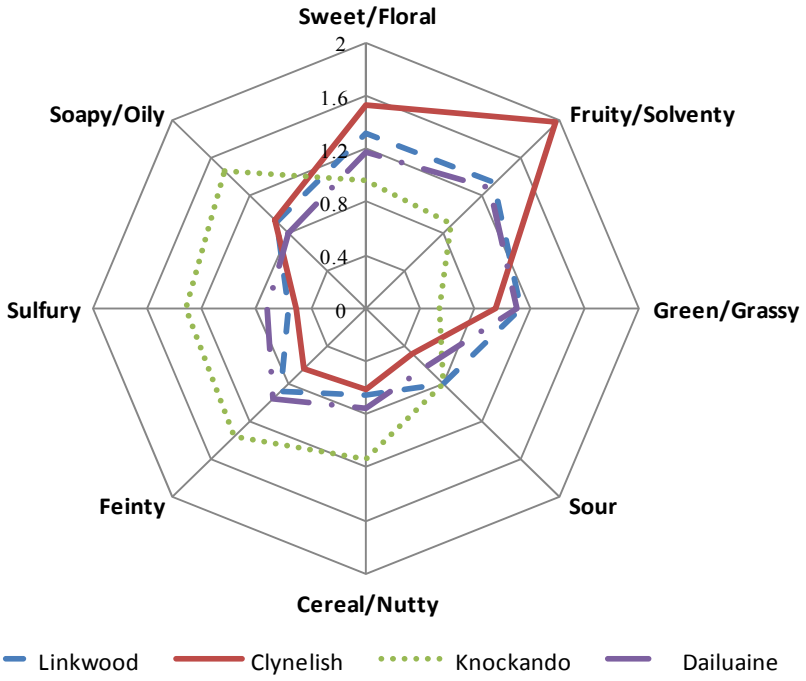


Figure 3.14 – Radar plot of aroma attributes for unpeated malt whiskies.

Table 3.18 showed significant difference in terms of most of the aroma attributes, with the exception of sweet/floral and sour aromas. The aroma profile of Knockando was significantly different from the other samples, having intense aromas of soapy/oily, sulphury, feinty and cereal/nutty and less green/grassy and fruity/solventy notes. Clynelish was characterised by a dominant fruit/solventy aroma.

QDA of unpeated malt – Test 2

The second set of unpeated malt whiskies were Glendullan, Cardhu, Blair Athol and Benrinnes. Results are shown in Table 3.19 and Figure 3.15.

Table 3.19 – QDA scores of aroma attributes for unpeated malt whiskies and ANOVA, using whisky types as a factor.

Aroma Attribute	Glendullan	Cardhu	Blair Athol	Benrinnes	p-value
Sweet/Floral	1.6	1.6	1.5	1.3	0.656
Fruity/solventy	1.7	1.8	2.0	1.1	0.007*
Green/Grassy	1.2	1.4	1.5	0.7	0.003*
Sour	1.0	0.8	0.9	0.9	0.704
Cereal/Nutty	1.1	1.1	0.9	1.1	0.778
Feinty	0.9	0.9	0.7	1.4	0.003*
Sulphury	0.7	0.6	0.4	2.0	0.001*
Soapy/Oily	0.7	0.8	0.9	0.9	0.627

**p-values < 0.05 showing significant differences of aroma attributes between four unpeated malt whiskies*

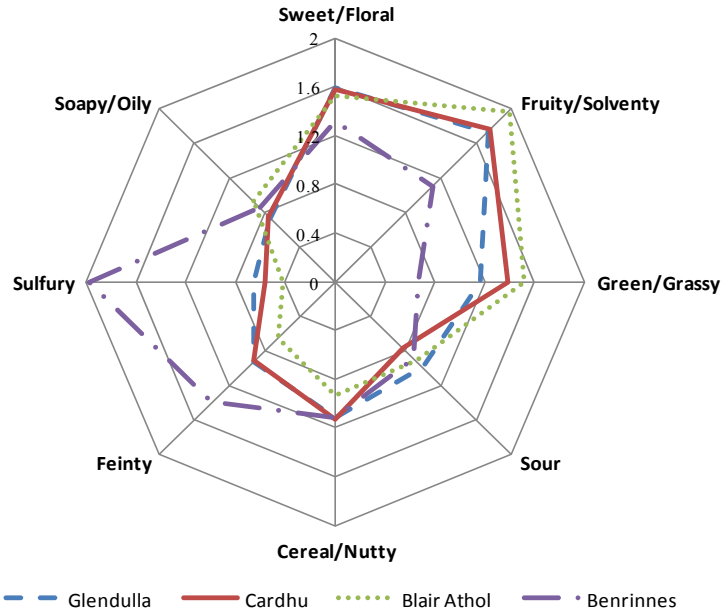


Figure 3.15 – Radar plot of aroma attributes for unpeated malt whiskies.

Table 3.19 showed differences between the unpeated malts in terms of half of the aroma attributes (i.e. green/grassy, fruity/solventy, sulphury and feinty). The main reason for these was due to Benrinnes, which had an aroma profile quite different from the others with dominant sulphury and feinty aromas. Glendullan, Cardhu and Blair Athol were more

similar to one another having dominant fruity/solventy aromas.

3.3.2.3 Aroma interaction measurement for unpeated malts

The aroma interaction capacities of each of the eight unpeated malt whiskies were measured using both a scaling test and by threshold measurement.

AIC by scaling

The overall peaty intensity was examined the panel scores were analysed statistically to evaluate differences between the samples (Figure 3.16).

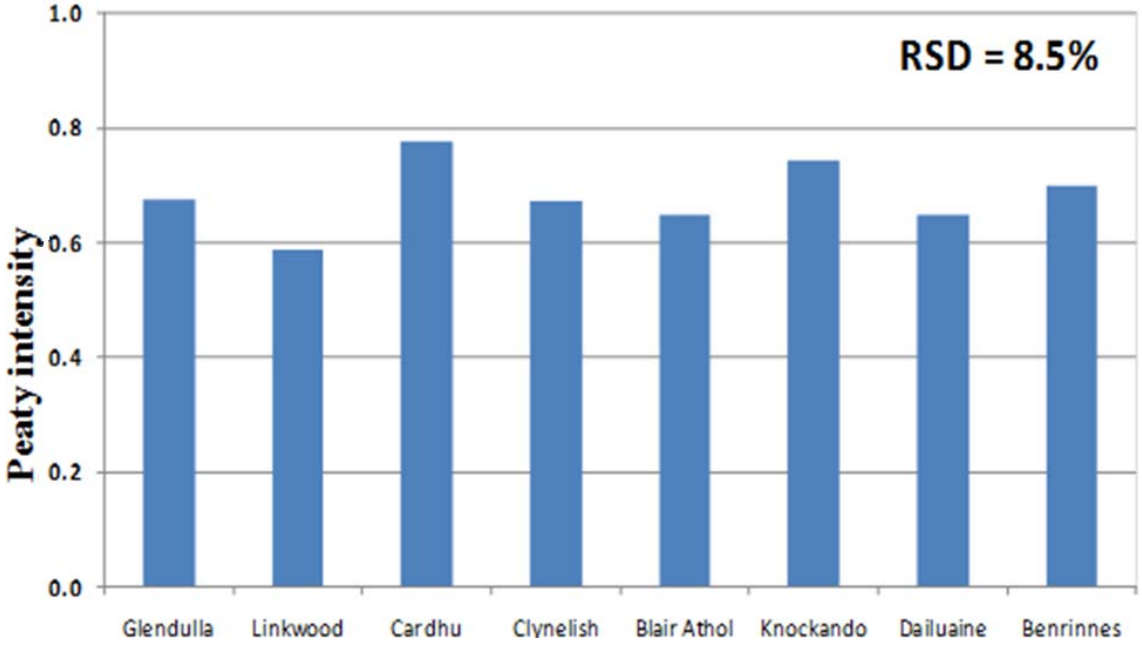


Figure 3.16 – Sensory scores for the overall peaty intensity for unpeated malt based blends.

ANOVA showed no significant (p-value = 0.4206) difference in overall peaty intensity and a relatively small variation between the samples.

AIC by threshold measurement

Threshold of peated whisky in the eight unpeated malts are shown in Figure 3.17.

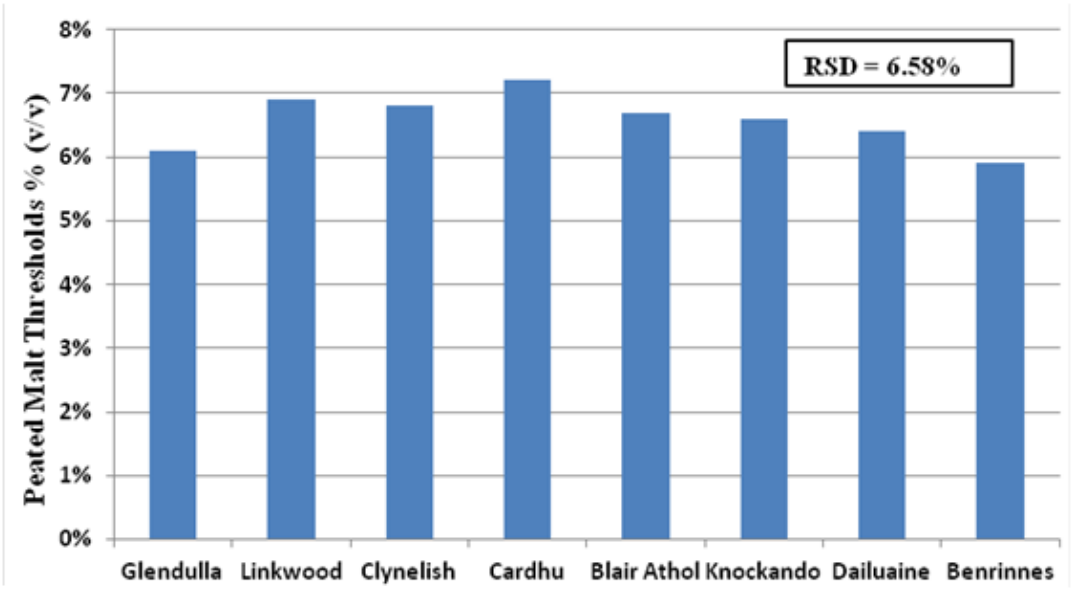


Figure 3.17 – Threshold levels of peated malt in the unpeated malt whiskies

There are no statistically significant (p-value=0.698) differences in the peated malt thresholds between the matrices (with an average of 6.6% variation). A similar threshold was observed in earlier experiments in Chapter 3.1.1.1 for vatted malts (6% (v/v) peated malt). Therefore, in practice, the differences in the degree of the aroma interaction capacity between the malts are unlikely to be an important factor. Although the unpeated malt whiskies varied in composition and sensory character, this variation did not affect their ability to mask peaty character.

3.3.3 Aroma Interaction Capacity comparison between standard grain and unpeated malt whiskies

Aroma Interaction Capacities for the standard grain and unpeated malt whiskies, determined by both scaling and threshold measurement, and were compared in Figure 3.18 and Figure 3.19. Although the scaling test is not strictly quantitative, the comparison still gives some indication for the AIC difference between grain and malt whiskies. Threshold measurements are more quantitative and give a more accurate indication of the degree of difference between the grain and malt samples (Lawless and Heymann 1998a).

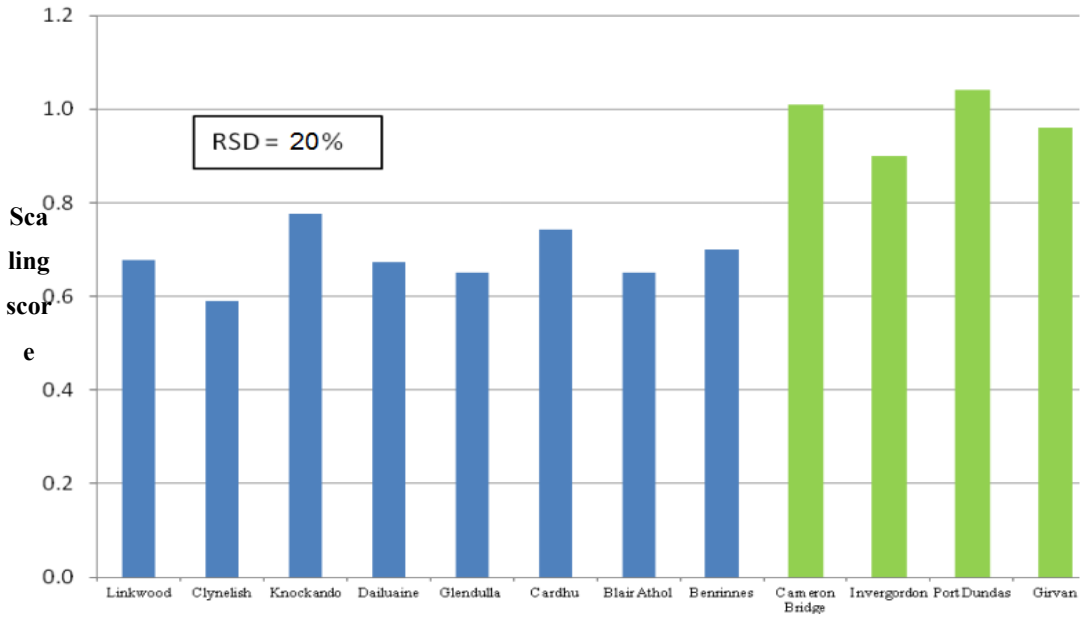


Figure 3.18 – Overall peaty intensity by scaling comparing grain (green) and malt (blue) whiskies.

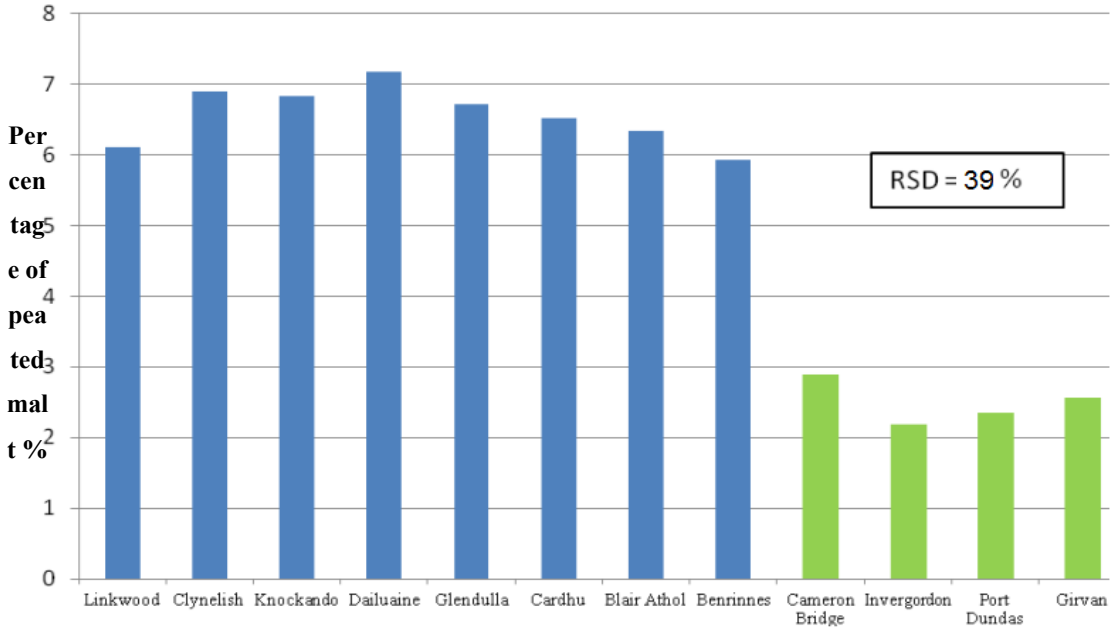


Figure 3.19 – Thresholds of peated whisky in grain (green) and malt (blue) whiskies.

Both sensory approaches showed a clear difference in the AICs between the standard grain and unpeated malt whiskies.

3.3.3.1 Chemical profile comparison

Generally speaking, the total major congener content in malt samples were showing higher score comparing with grain samples, malts total major congener content was about two to three times higher than grains (Tables 3.3.1 and 3.3.3). The total trace congener content between grain and malt blend samples was massive, as all the grain samples score were below 10 mg/L, whereas malt got average score above 90 mg/L for total trace congener content. Chemical composition was compared statistically with the Scaling and Threshold measured aroma interaction. The resulted R^2 is shown in Table 3.20.

Table 3.20 – Output of linear regression analysis of distillate congeners and aroma interaction.

Analysts	Scaling	Threshold
	R^2 value	
(D1) acetaldehyde	0.72*	0.80*
(D2) ethyl acetate	0.62*	0.72*
(D3) acetal	0.68*	0.64*
(D4) methanol	0.64*	0.48*
(D5) n-propanol	0.23	0.38*
(D6) iso-butanol	0.22	0.43*
(D7) iso-amyl acetate	0.62*	0.73*
(D8) n-butanol	0.73*	0.85*
(D9) 2-methyl-1-butanol	0.68*	0.87*
(D10) 3-methyl-1-butanol	0.78*	0.93*
Total Major	0.76*	0.868*
(D11) ethyl hexanoate	0.67*	0.85*
(D12) ethyl octanoate	0.64*	0.85*
(D13) ethyl decanoate	0.66*	0.81*
(D14) ethyl dodecanoate	0.51*	0.65*
(D15) ethyl tetradecanoate	0.59*	0.71*
(D16) ethyl hexadecanoate	0.77*	0.83*
(D17) ethyl 9-hexadecenoate	0.27*	0.36*
(D18) 2-phenethyl acetate	0.53*	0.58*
(D19) 2-phenethyl alcohol	0.54*	0.57*
Total Trace	0.86*	0.89*

**p-values < 0.05 showing significant correlations between the chemical compounds and the aroma interaction*

As mentioned in Chapter 3.3.1 and Chapter 3.3.2, the standard grain and the unpeated malt have a relatively consistent degree of the aroma interaction between different distilleries,

and the proportion of these two types of whisky is found to have a relatively big impact on the peaty aroma interaction in the blend. The significant correlations between distillate congeners and aroma interaction are found mainly due to the distillate congeners which are thought to be good indicators to distinguish the proportion of the grain and the malt whiskies.

Table 3.20 suggests that 3-methyl-1-butanol is a good marker for AIC. However, as previously found in Chapter 3.3.1 all the grain whiskies have similar degree of AIC, same phenomena also been found in the malt study Chapter 3.3.2. Therefore, 3-methyl-1-butanol has good ability to indicate the AIC not because of its sensory property but it is a good marker for malt content. In addition, traditionally the total amyl alcohols (i.e. 2 and 3-methyl-1-butanol) are commonly used in Scotch whisky authenticity analysis for measuring the malt contents in whisky blends(Aylott et al. 1994; Lee et al. 2001a; Aylott 2003; MacKenzie and Aylott 2004) since the levels of amyl alcohols are usually more consistent in malt whisky than in grain whisky (Table 3.6).

Nowadays it is still very hard to use single or a cluster of congeners for representing whisky aroma. Consequently, it is not surprising that in this study using chemicals to represent the aroma interaction capacity will be challenging. On the basis of this data, it seems that the chemical congeners are generally good indicators for distinguishing between malt and grain but for the interpretation of the variation of AIC, the levels of the congeners themselves are not sufficiently diagnostic. To further explore the relationship between congeners and AIC data, this will be explored on a sensory basis in Chapter 3.3.4.

3.3.3.2 Sensory comparison between standard grain and unpeated malt

Previous sensory QDA test results were also compared in Table 3.7, to help try to understand the relationship between sensory profile and whisky AIC. As expected, malt sample were generally high in all the attribute scores.

As previously introduced in Chapter 3.1.3, if the aroma interactions in whisky are mainly due to physiological interactions, then all of these aroma attributes should more or less contribute to, and influence, the aroma interaction. This suggests that the overall intensity

of the aroma score (sum of all the attributes) should give the best index to indicate the presence of aroma interactions. To further explore the relationship between aroma intensity and AIC, and test the principle of the aroma interaction, the relationship between the distillate aromas, Scaling and Threshold measured aroma interactions were tested, by applying linear regression analysis. The resulting R^2 values are shown in Table 3.21.

Table 3.21 – Output of linear regression analysis of QDA aroma data and aroma interaction data.

Aroma attributes	Scaling	Threshold
	R^2-value	
Sweet/Floral	0.64*	0.73*
Fruity/solventy	0.57*	0.68*
Green/Grassy	0.54*	0.64*
Sour	0.45*	0.38*
Cereal/Nutty	0.47*	0.61*
Feinty	0.39*	0.41*
Sulphury	0.04	0.02
Soapy/Oily	0.44*	0.52*
Overall intensity	0.80*	0.83*

**p-values < 0.05 showing significant correlations between the aroma attributes and aroma interaction*

It is shown in Table 3.21 that the majority of the aroma attributes show significant correlations with both Scaling and Threshold measured aroma interaction, except for sulphury aroma as expected. It was also found in Table 3.21 that the overall aroma intensity is better represented the AIC compared with any other single aroma attribute. Thus the overall aroma intensity generated the highest correlation with both Scaling and Threshold measurement. This indicated once again that the aroma interactions in whisky blends are mainly influenced by physiological interactions. The aroma interaction capacity is highly influenced by the aroma intensity of the hosting matrices. Grains are generally considered to be weaker matrices in terms of aroma intensity, with consequently lower AIC compared to malt matrices.

3.3.4 Summary

Overall aroma interactions observed appear to be related to the hosting matrix aroma

intensity (physiological interaction), and the aroma intensity of a matrix is in turn highly influenced by its chemical composition. In other words, the aroma congeners give the matrix itself aroma, and it further impacts on the aroma interaction, so all these factors are dynamic related and none of them exist independently. Especially, as shown in Table 3.21, the overall matrix aroma intensity is highly related with its aroma interaction capability.

All the relevant results showed that grain whiskies have very similar overall aroma intensity, although some of samples were sweeter and some got more sulphur note, but overall the grain whiskies were on the same aroma intensity magnitude. A similar phenomenon was found within the malt study, all eight tested malts were giving much closer overall aroma intensity score. It is very useful and important finding for master blender to create their new whiskies, during the blending practice if they need consider the aroma interaction (or masking effect), the proportions of the grain and malt are more important factors to impact on aroma interaction capacity in a blend, rather than any minor differences of samples between distilleries. This implies that without consideration of other factors in a blend (such as peaty and woody intensity), the most important parameter to impact on the peaty aroma perception is the proportion of malt and grain used.

Another point to note, observed from Table 3.20-Table 3.21, is that threshold-based AIC seems to be a promising parameter, which significantly correlated with chemical and sensory data. Also the nature of the threshold measurements themselves works as linkages between chemical concentration and aroma intensity (peaty intensity). By using threshold AIC, it may possible to create a model to predict the outcome of the peaty aroma interaction.

3.4 Aroma interaction capacity study for woody grain whiskies

Five woody grain whiskies were sourced from Cameron Bridge distillery (Table 2.5) which had been matured for 2, 4, 7, 9 and 12 years in first fill ex-bourbon casks. Chemical and sensory differences were also explored and the relationships between these and the AIC of each sample studied.

3.4.1 Chemical profile comparison of the woody grain whiskies

The levels of maturation-derived compounds were determined using HPLC and statistical analysis applied to assess differences between the samples. Results are shown in Table 3.22.

Table 3.22 – Mean (three analyses) concentrations of maturation-derived compounds (mg/L) with % RSD for the woody grain whiskies and ANOVA

First-fill Ex-bourbon Cameron Bridge (mg/L)						
Analyte	**2 years**	**4 years**	**7 years**	**9 years**	**12 years**	**p-value**
	Mean (%RSD)					
(W1) gallic acid	3.3 (0.2)	2.7 (1.7)	6.7 (1.0)	6.3 (0.7)	5.0 (1.3)	< 0.05
(W2) ellagic acid	11.4 (0.2)	13.9 (0.2)	14.4 (0.1)	18.7 (0.6)	16.5 (0.1)	< 0.05
(W3) coniferaldehyde	1.8 (1.6)	2.1 (0.4)	0.8 (1.0)	1.0 (1.1)	1.0 (2.0)	< 0.05
(W4) vanillin	2.2 (3.3)	3.2 (0.8)	3.4 (0.8)	3.8 (1.0)	3.8 (0.5)	< 0.05
(W5) vanillic acid	0.8 (3.3)	1.2 (2.3)	1.1 (2.0)	1.5 (2.7)	2.5 (0.5)	< 0.05
(W6) sinapaldehyde	3.8 (1.0)	3.9 (0.2)	1.2 (1.6)	1.3 (2.5)	1.8 (1.3)	< 0.05
(W7) syringaldehyde	6.3 (0.7)	8.4 (0.2)	5.7 (1.4)	7.0 (1.2)	7.9 (0.7)	< 0.05
(W8) syringic acid	1.4 (0.3)	2.1 (3.5)	1.8 (1.4)	2.3 (0.7)	4.3 (3.0)	< 0.05
(W9) scopoletin	0.6 (10.3)	0.9 (5.8)	1.2 (6.1)	1.4 (2.3)	0.9 (4.2)	< 0.05
(W10) 5-HMF	1.9 (1.2)	1.7 (1.9)	0.9 (1.8)	0.8 (3.4)	0.7 (1.8)	< 0.05
Total	33.5	40.1	37.2	44.1	44.4	< 0.05

**p-values < 0.05 showing significant differences between the whiskies*

Table 3.22 showed significant differences in the levels of all of maturation-derived compounds between the samples ($p < 0.05$). The chemical composition remained relatively consistent in this test. First fill ex-bourbon casks, as studied here, give high levels of wood derived compounds, with a rapid extraction in the first 6-12 months of maturation. It was

observed that the maturation-derived compounds did not show a clear increase in concentration with increasing maturation time. These grain whiskies had been sampled from different casks and the maturation-derived compounds are highly affected by individual cask parameters, such as variation in the initial heat treatment. The alternative, to sample at time intervals from the same cask, was not feasible however due to the maturation periods involved (Nishimura and Matsuyama 1989; Conner et al. 2003).

Prolonged maturation did give an increase in woody congener content. The two year old whisky contained 33.5 mg/L total wood derived congeners, while the 12 year old contained 44.4 mg/L. Also the levels in all of these samples were much higher than those found in the previously tested standard grain (14.2 mg/L), unpeated malt (10.2 mg/L) and peated malt (9.3 mg/L), which had all been matured in re-fill casks for three years.

3.4.2 Sensory comparison of the woody grain whiskies

QDA data was collected for the five woody grain whiskies using four aroma attributes related to woody character: dried fruity, spicy, sweet and overall woody intensity and ANOVA performed to determine any significant differences between the samples. Results are shown in Table 3.23.

Table 3.23 – QDA scores for the woody grain whiskies

Aroma attributes	Maturation time (year)					p-value
	2	4	7	9	12	
Dried fruity	0.9	1.0	1.1	1.1	1.3	0.0871
Spicy	0.7	1.0	0.9	1.1	1.1	0.1430
Sweet	0.9	1.0	1.1	1.2	1.1	0.5865
woody	1.2	1.1	1.0	1.2	1.8	0.0036*

**p-values < 0.05 showing significant differences of aroma attributes between different aged woody grain whiskies*

Only the overall woody intensity showed a significant difference between the samples. However, it did not show a clear trend with maturation time.

3.4.3 Aroma Interaction Capacities of the woody grain whiskies

The aroma interaction capacities of each of the five woody grain whiskies were measured using both a scaling test and by threshold measurement.

3.4.3.1 AIC by scaling

Figure 3.20 shows the sensory scores for the overall peaty intensity levels for 10% (v/v) peated malt in each of the woody grain whiskies.

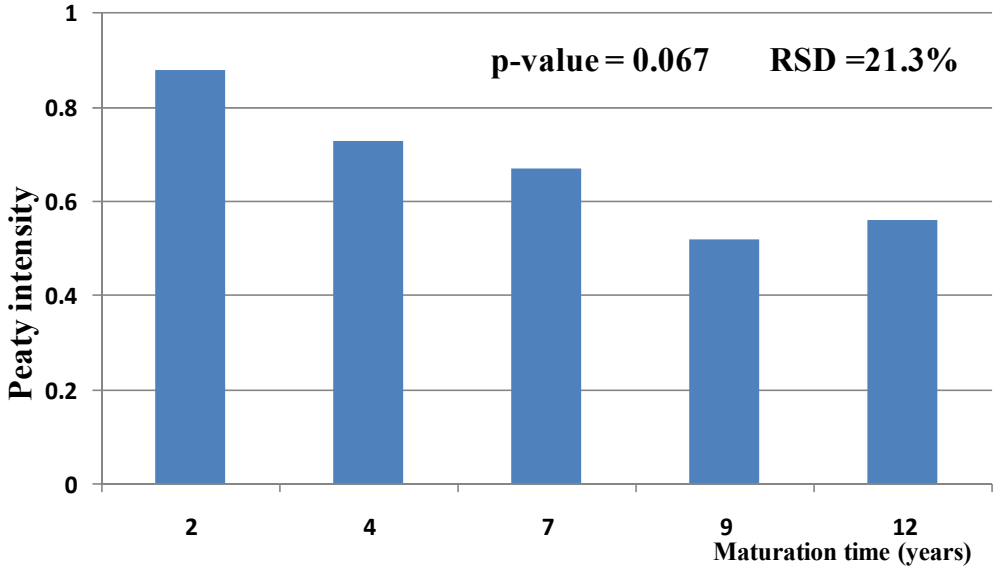


Figure 3.20 – Sensory scores for the overall peaty intensity for the woody grain whiskies.

Compared with the results observed for the standard grain and the unpeated malt (Chapter 3.3) a relatively high RSD (21.3%) was found suggesting a higher degree of variation between the samples. There was a linear decrease in overall perceived peaty intensity from the 2 year old through to the 9 year old, but this trend did not continue at 12 years maturation. This may because the maturation effect will continue decrease through the year increase. For instance, there is no significant difference between 9 and 12 year old and that maybe because a suppression maximum had been reached, and this is supported by the following test in Figure 3.21.

3.4.3.2 AIC by threshold measurement

Threshold of peated malt in the five woody grain whiskies are shown in Figure 3.21.

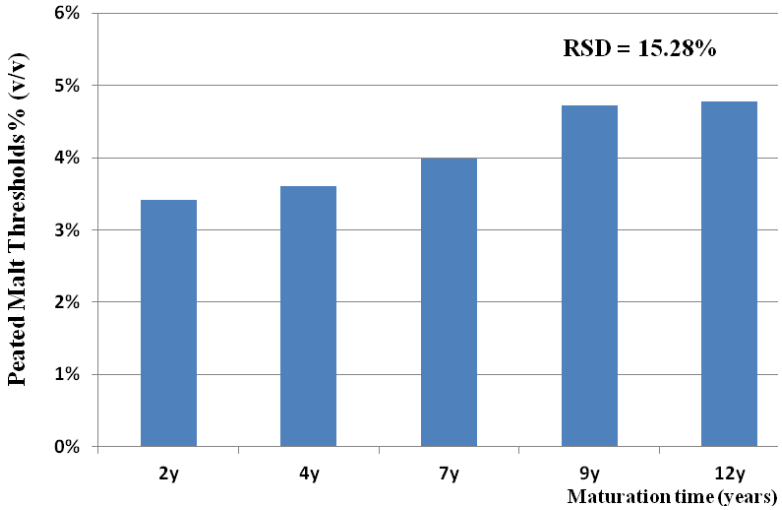


Figure 3.21 – Threshold levels of peated malt in the woody grain whiskies.

The thresholds of the peated malt clearly increased with maturation time. This increase suggests that the impact of woody character and its related aroma interaction on peaty character exists.

3.4.4 Linear Regression Analysis

Linear regression analysis was performed to identify the relationship between the maturation-derived compounds, woody aroma attributes and its realted aroma interaction capacity. Data obtained from the Table 3.22Table 3.23 and Figure 3.20Figure 3.21

3.4.4.1 Links between wood related aromas and AIC

Correlations between individual aroma attributes and AICs are shown in (Table 3.24).

Table 3.24 – Output of linear regression analysis of aroma attributes and AICs.

Aroma attributes	Scaling	Threshold
	R-squared	
Woody	0.23	0.46
Dried fruity	0.75	0.86*
Spicy	0.88*	0.71
Sweet	0.80*	0.53
Overall intensity	0.69	0.82*

**p-values < 0.05 showing significant correlations between aroma attributes and AICs*

None of the aroma attributes showed significant correlations with both scaling and threshold AICs.

3.4.4.2 Links between maturation-derived compounds and AICs

Correlations between each of the maturation derived compounds and the AICs are shown in Table 3.25. The majority of the maturation-derived compounds showed no significant correlations with AIC ($p > 0.05$). Exceptions were ellagic acid, vanillin, 5-HMF and the total level of maturation-derived congers, which all showed significant correlation for both scaling and threshold measured aroma interaction. It is believed, those that did not correlate may not be sensory significant to woody character.

Table 3.25 – Output of linear regression analysis of maturation-derived compounds and AICs (at 5% significance level).

Analytes	Scaling	Threshold measurement
	R-value	
(W1) gallic acid	-0.669	0.651
(W2) ellagic acid	-0.974*	0.922*
(W3) coniferaldehyde	0.687	-0.736
(W4) vanillin	-0.978*	0.880*
(W5) vanillic acid	-0.744	0.828
(W6) sinapaldehyde	0.800	-0.798
(W7) syringaldehyde	-0.257	0.184
(W8) syringic Acid	-0.656	0.758
(W9) scopoletin	-0.820	0.671
(W10) 5-HMF	0.917*	-0.916*
Total congener	0.923*	0.878*

**p-values < 0.05 showing significant correlations between maturation derived compounds and AICs*

3.4.5 Summary

Based on the above data, the woody related aroma interaction capacity is related to the aroma intensity of the woody grain matrix, and some wood-related aroma congeners. Unlike distillate character, wood-related compounds and aroma character are both caused by a single process, wood cask maturation. Although the compounds measured may not

fully represent woody character, they still provide a good indicator. Overall, the AIC measurement by both scaling and threshold measurement showed an increase with maturation time (Figure 3.20Figure 3.21). Both methods show a good relationship with chemical and sensory data, but the threshold method gave better correlation results. This suggested the possibility of using such threshold measurements as parameter for the next stage, namely the development of a prediction model.

3.5 Development of models to predict aroma interactions during whisky blending

The major objective of this study was to develop feasible approach to predict aroma interactions during whisky blending, in order to give more scientific and analytical support in the art of whisky blending. Measurement and comparison of the aroma interaction capacities of different whisky types on peaty character have been discussed previously (Chapter 3.1.1). Prediction models were developed based on the findings of these earlier experiments. All models investigated in this study are based on Odour Unit calculate Equation 1 (Chapter 1.5.2.3).

3.5.1 Development of a prediction model based on phenols thresholds

Peaty character response was found to be influenced by the types of the whisky used in blending in previous experiments. Therefore, the next stage of this study was to try to quantify the matrix effect using both analytical and sensory approaches, and then to determine correlations between peaty sensory responses of blended whisky with analytical data.A phenol threshold based model was then created to give the prediction of peaty intensity for a particular blend. Three types of whiskies (Grain, Woody and Malt) were applied as different matrix backgrounds. Eight phenols, namely phenol, ethyl phenol, o-cresol, p-cresol, m-cresol, guaiacol, 4-methyl guaiaol and 4-ethyl guaiacol have been used as a stimulus to induce peaty character in blended samples.

3.5.1.1 The calculation of the predicted sensory response

In this study, the single compound stimulants used were individual phenols, and the odour unit of peaty aroma calculated by applying Equation 2.

$$\boldsymbol{Odour\ Unit\ (O) = \frac{phenol\ concentration\ (C)}{phenol\ threshold\ (FIC)}} \qquad \textbf{Eq. 2}$$

Where C is phenol concentration and AIC is the phenol threshold value in a particular matrix (mg/l). The Odour intensity (OI) of one $phenol_n$ in particular blended samples, can be expressed as the OU value times volume percentage (V%) of the particular matrix solution used in blended samples. In this study the three matrices (Grain (G), Woody (W) and Malt (M)) were used, and the odour intensity of $phenol_n$ expressed as Equation 3:

$$Odour\ Intensity\ (OI_n) = (OU_g \times V_g\%) + (OU_w \times V_w\%) + (OU_m \times V_m\%) \quad \textbf{Eq. 3}$$

Finally the prediction of overall peaty intensity of particular blended samples can be calculated as the sum of all phenols odour intensity and express as Predicted peaty intensity in Equation 4:

$$Predicted\ peaty\ strength = \sum_{k=1}^{n}(OI)_1\ V_1\% \quad \textbf{Eq. 4}$$

The previously measured phenol concentrations and phenol thresholds in Chapter 3.1 were used as an evaluation test for this phenols based prediction.

3.5.1.2 Evaluation of phenol based prediction model

In this phenol based prediction, by combining the individual threshold data (Table 3.7) and headspace phenols concentration data (Table 3.8), the odour units for each of the three blends (grain, woody and malt) were calculated based on Equation 3.

Table 3.26 – Odour units of each of the phenols based on the headspace concentrations and threshold (AIC)

Matrices	Standard grain blend	Woody grain blend	Unpeated malt blend
4-ethylguaiacol	1.6	1.0	0.5
4-ethylphenol	0.2	0.1	0.2
guaiacol	4.3	2.5	1.8
m-cresol	0.4	0.3	0.2
4-methylguaiacol	0.1	0.1	0.0
o-cresol	0.6	0.4	0.2
p-cresol	3.0	1.3	1.6
phenol	0.0	0.0	0.0
Total	10.2	5.6	4.6

It was found that the total odour units in the three blends varied considerably (Table 3.26), even though all three blends contained similar levels of phenols in the headspace (Table 3.7). The standard grain blend had the highest total odour units, while odour units for the woody grain and unpeated malt blends were about half of the grain blend.

It was also found that although phenol itself makes up the biggest proportion of the total phenolic compounds (Table 3.8 and Table 3.26) it only makes a very small contribution in

terms of odour intensity. Guaiacol, p-cresol and 4-ethylguaiacol give about 90% of the contribution to the total odour units, but are less than 10 % of the total phenol concentration. This suggests that the most abundant phenolic compounds in whisky do not have much aroma impact, while more minor phenols, in quantity terms, give the biggest odour contribution due to their relatively low sensory thresholds. For better comparison, the observed and calculated peaty intensities and analytical measures of phenolic compounds are shown in Table 3.27.

Table 3.27 – Comparison of observed peaty intensity, total odour units and analytical levels of total phenols concentration in the three blends.

Matrices	Standard grain blend	Woody grain blend	Unpeated malt blend
Observed peaty intensity	1.4	0.7	0.7
Total odour units	10.2	5.6	4.6
Total phenol concentration	1.2	1.2	1.1

In terms of perceived peaty intensity the standard grain blended was twice as peaty as the unpeated malt and woody grain blends. However, if just considering the analytical data (Total phenol concentration), the three blended samples should have had a similar intensity of peaty aromas. In ratio terms the total odour units were much closer to the observed sensory response than the analytical data, though in numerical value terms they are quite different. Clearly, using total odour units to predict of peaty character is much more predictive than simply using the analytical data.

3.5.1.3 Validation using different peated malt concentrations

The previous tests demonstrated the ability of phenols model to predict the matrix effect on peaty character when a fixed amount of peated malt was present (10% (v/v) Caol Ila). To further test the threshold based model, an additional experiment was designed to test different levels of peated malt addition in different matrix background, namely 5% (v/v) in standard grain and 10% (v/v) in unpeated malt. Samples were evaluated for the peaty intensity by three methods, included observed peaty intensity, total odour units and traditional analytical measurement of total phenols (Table 3.28).

Table 3.28 – Comparison of observed peaty intensity, total odour units and analytical levels of Total phenol concentration in blends produced using different concentrations of peated malt.

Matrices	5P95G *	10P90M#
Observed peaty intensity	0.5	0.3
Total odour units	5.4	4.6
Total phenol concentration	0.6	1.1

***5P95G:** 5% peated malt + 95% standard grain*
*#**10P90M:** 10% peated malt + 10% unpeated malt*

The traditional analytical based method did not provide a model that corresponded to observed peaty intensity. Based on the phenol levels the 5P95G should have about half of the peaty intensity of the 10P90M. However, in reality the 5P95G had double the peaty response, again showing strong evidence of the presence of the aroma interactions during blending. The phenol based prediction model was able to predict the intensity relationship between 5P95G and 10P90M, although the prediction values are not in the same numeric range as observed.

3.5.1.4 Summary

This study demonstrated, by measuring the phenol thresholds in different matrices, that it was possible to quantitatively measure the aroma interaction capacity, thereby associating phenols concentrations with the overall sensory response. Furthermore, the concept of odour intensity was used, which was a mathematical approach to predict the peaty sensory response. A correlation was found by comparison the predicted sensory response (predicted peaty strength) with the observed sensory response. Although, the predicted values are not in the same magnitude as observed, it is still more meaningful than traditional phenol measurement prediction.

3.5.2 Development of a prediction model based on potent aroma marker compounds

The existence of aroma interactions imply "antagonistic or suppression effects" in the whisky blends that influence the perception of peaty character (Conner et al. 1994; Conner et al. 1998). Previous Chapters (Chapter 3.1.4) studied these aroma interactions in various whisky bases, from a sensory and an analytical perspective. These previous studies concluded that the intensity of peaty aroma character was strongly related to the aroma

interaction capacity of the matrix background. It is hypothesised that such interactions are due to the presence of different key aroma compounds in the various whisky matrices that mask the phenolic compounds responsible for peaty aromas. It is reported in Chapter 3.1.5 that the main reason for the occurrence of aroma interactions in blended whisky is likely to be due to physiological matrix interactions. This implies that the greater the aroma intensity of the matrix, the larger the aroma interaction and effect on peaty perception.

Therefore, in this study a new approach was explored in which these potent aromatic compounds (marker compounds) were identified and their sensory influence examined. This approach was adapted from the research strategy reported by Boscaini *et al.* (2003; GC-O introduction in Chapter 1.5.1.4). Marker compounds were identified and quantified for each whisky base. A model was then developed based on these levels and its ability to predict peaty perception tested in different whisky matrix types.

3.5.2.1 Identification of marker compounds by Gas Chromatography-Olfactometry aroma extract dilution analysis

Gas Chromatography-Olfactometry Aroma Extract Dilution Analysis (GC-O AEDA) was used to identify the most potent aroma compounds in each whisky matrix that could subsequently be used as markers. In general, the identification of 'important' or 'of the most impact' odour compounds in a food matrix is based on odour activity value (OAV). The use of GC-O facilitates the process of determining OAVs while GC-O AEDA is used to clarify the most active odour compounds (Blank 1996; Friedrich and Acree 2000). The principle of this analysis is based on 'dilution-to-threshold'. As the sample is sequentially diluted the number of odours detected by the panellists decreases, as the concentrations of the congeners in the sample are diluted to below their detection thresholds. The remaining odour compounds detected in the most dilute samples are identified as the most potent aroma compounds.

As detailed in the GC-O methodology (Chapter 2.4.1), four panellists assessed each sample. The two types of detection odour categories been summarized in Table 3.29. The number of total detected odours decreased as the dilution factors increased (e.g. Unpeated malt 1/1=118 > 1/10=65 > 1/100=30). However, the number of the detected odours for each

aroma category did not always decrease as the dilution factors increased (e.g. Unpeated malt floral 1/1=12 < 1/10=16). This could be caused by a number of factors, such as mis-description or an odour being counted more than once and some compounds also change descriptor depending on their concentration. During GC-O assessment, the odour intensity is generally weak and often only appears for a few seconds within the sniffing port. This makes it difficult for the panellist to catch every odour and selected the accurate descriptor for each odour. Also some of the panellists may be particularly sensitive to an odour during the GC-O test (low detection thresholds), but may have a much higher average threshold compared to other panellists. To reduce the misdetection and mis-description influence, the total confirmed identified odours were introduced. Here an odour was only counted if it had been detected by at least three panellists, giving more robustness to an identification that represented the majority of the sensory assessors' threshold levels.

Table 3.29 – Summary of GC-O AEDA results for each whisky matrix type.

Whisky sample / Sensory character	Standard grain			Unpeated malt			Woody grain		
	1/1	1/10	1/100	1/1	1/10	1/100	1/1	1/10	1/100
Floral	18	7	6	12	16	2	25	7	16
Sweet	23	14	5	14	12	7	29	30	13
Fruity	13	4	1	20	3	6	14	4	2
Vegetable	3	1	0	2	4	0	5	1	1
Grassy	1	2	0	7	4	1	7	2	2
Sour	0	0	0	1	0	0	1	0	0
Cereal	0	1	0	3	0	2	1	2	1
Nutty	4	2	0	2	2	2	3	5	1
Stale	4	3	1	8	2	2	3	1	1
Sulphury	1	1	0	1	2	0	0	2	1
Oily	0	0	0	10	3	1	0	0	0
Feinty	0	0	0	0	0	0	0	0	0
Smoky	5	4	1	12	4	0	7	3	1
Burnt	4	0	0	3	5	0	8	0	0
Medicinal	5	5	3	13	1	2	5	8	4
Spicy	0	2	0	0	0	0	0	5	3
Solventy	14	3	0	8	1	0	9	4	2
Other	9	1	2	2	6	5	18	17	2
Total detected odours	**104**	**50**	**19**	**118**	**65**	**30**	**135**	**91**	**50**
Total confirmed identified odours	**13**	**4**	**1**	**12**	**4**	**3**	**11**	**4**	**4**
Average odour intensity	**1.50**	**1.35**	**1.30**	**1.65**	**1.43**	**1.30**	**1.55**	**1.53**	**1.46**

GC-O analysis revealed many odorous compounds in three different whisky matrices, but only a few of them were identified as potent compounds (Table 3.29). For example, in the standard grain 104 odours were observed in the 1/1 dilution, but only 12 of them were confirmed identified. Only one of these remained after a 100-fold dilution. With dilution there was not only the expected reduction in the number of odour compounds, but also a decrease in the average odour intensity. We classified five dominant odours, *floral, sweet, fruity, solventy* and *other* (other is the aroma, which cannot be categorized into any pre-setup aromas) were detected in the analysis, comprising of over 50% of the odours detected across all sample types.

The performance and sensitivity of individual panellists varied greatly, with individual panellist detecting some odours which could not be detected by the others. This can be caused by differences in both sensory and describe abilities included:

1. Assessors differ in their sensitivity and thresholds for sensing individual components of character (Gregson 1962; Lee et al. 1999b; Sterckx et al. 2011).
2. Assessors may lack awareness or cognizance of certain sample attributes (Lee et al. 1999b).
3. Most describe vocabulary were generally used and defined in "complex whisky matrix" that are not easily identified or recognized in their single chemical form (GC-O test).

Due to the considerable difference of sensory perception of individuals, data was only accounted when the majority panellists (three out of four) detected the same odour character (Friedrich and Acree 1998; 2000). Therefore, the confirmed identified odours in 100-fold diluted samples were further evaluated individually for each whisky types in the following sections, to determine the feasibility of using these odour compounds as markers.

The number of detected odours was expectedly higher in unpeated malt and woody grain samples than in standard grain whisky, agreeing with the previous chemical analysis that demonstrated that the standard grain is relatively less complex (Chapter 3.3.1). The unpeated malt and the woody grain matrices contained a greater number of dominant odour compounds, i.e. compounds remaining in the 1/100 dilution. This can be observed in the number of the identified odours in all dilutions of each sample. The

standard grain samples had the least odours remaining at the 1/100 dilution. This phenomenon confirms once again that in a matrix, the chemical composition is proportionally related to its aroma intensity and magnitude of aroma interaction.

The previous sensory profiles generated for these three whisky matrices (Chapter 3.1) showed significant difference in terms of *Sour* and *Sulphury* aromas. However, in the GC-O test only a limited number of compounds were detected that exhibited these two attributes, and little difference was observed between the whisky matrices in Table 3.29. There are several possible factors might cause these differences between previous QDA and the GC-O data.

1. The sample is being presented in a different form; In the QDA test the whole sample was presented, while in the GC-O study the sample was resolved into simple chemical components
2. Differences in sensory methodology; in QDA test panellist have enough time to assess the sample and consider the best attributes to use during the test. But in the GC-O test panellist have a very limited time of to assess each odour
3. Odour concentration differences; in the QDA test the sample were presented at their natural concentration (after a 20% (v/v) abv dilution), but in the GC-O test, sample were firstly concentrated by liquid-liquid extraction, and then diluted to three different concentrations. This greatly influences odour perception for panellists, since odours will behave quite differently with different concentration.
4. The concentration and analysis procedures for GC-O could produce artefacts, i.e. compounds that are not in the original sample but arise as a result of sample preparation or measurement.
5. An Odour compounds present in different concentration will be perceived as different odour character.

Identification of a marker compound for standard grain whisky

Table 3.30 shows the confirmed identified odour compounds in the three different concentrations of the standard grain whisky and several other parameters were recorded:

- Time: the average starting time of each panellist detecting the aroma
- Intensity: calculated from the average intensity of the four panellists' scores.
- Number of panellists detecting the aroma:
- Sensory character: (not in order of importance)

Table 3.30 – Confirmed identified aromas in the standard grain whisky.

No	Time	Intensity	Number detecting aroma	Sensory character
Grain 1/1				
1	10.4	2.0	4	Floral / Sweet
2	12.3	1.7	3	Solventy/sweet
3	16.1	1.7	3	Solventy / Other
4	18.9	1.3	3	Burnt / Smoky
5	21.9	2.0	3	Solventy / Floral
6	23.0	2.3	4	Sweet
7	23.2	1.8	4	Fruit / Floral/Sweet
8	26.4	1.5	4	Other / Medicinal
9	27	1.7	3	Floral / Fruit / Sweet
10	27.1	1.3	3	Solvent / Stale
11	27.3	1.3	3	Floral / Fruit
12	28.2	1.3	4	Sweet / Medicinal / Burnt
13	29.5	1.3	3	Sweet / Floral / Burnt
Grain 1/10				
1	10.4	2.0	4	Floral / Fruit / Sweet
2	12.3	1.0	3	Solventy / sweet
3	22	1.3	3	Sweet / Floral
4	23.3	1.3	3	Floral / Sweet
Grain 1/100				
1	10.4	1.8	4	Floral / Other / Sweet

Only one odour was still detected in the 100 fold dilution of the standard grain whisky. This compound is therefore believed to be the most potent odour in this matrix. It had a retention time of 10.4 min, and a *floral*, *sweet* aroma. With the use of all the collated information, the MS Spectral database strongly suggested that this compound was 3-methyl-1-butanol (m/z: 55/70, 85% match the reference library). The pure form of the compound was then tested by GC/MS-O direction injection, confirming its retention time and related aroma characters. 3-methyl-1-butanol is one of the most common higher alcohols appearing in alcoholic drinks as by-products of alcoholic fermentation (Aylott 2003; Dolan 2003). It is described as having a banana, alcohol, sweet and aromatic aroma, with a threshold of 70 mg/l in beer (Meilgaard 1975), which agrees with the description obtained in the GC-O tests.

Identification of a marker compound for unpeated malt whisky

Table 3.31 shows the confirmed identified odours in the three dilutions of the unpeated

malt.

Table 3.31 – Confirmed identified aromas in the unpeated malt whisky.

No	Time	Average intensity	Number detecting aroma	Sensory character
Malt 1/1				
1	10.6	2.3	4	Solventy / Oily / Cereal / Stale
2	11.2	2.0	4	Fruit / Sweet / Solventy
3	15.1	1.8	4	Fruit / Sweet / Stale
4	17.2	1.7	3	Oily / Solventy / Fruit
5	19.1	1.0	3	Smoky / Solventy
6	20.5	2.3	4	Nutty / Other / Solventy / Fruity
7	22.0	2.3	3	Fruit/ Sweet
8	23.3	2.8	4	Floral / Fruit
9	24.2	2.3	4	Grassy / Other / Stale / Sweet
10	27.0	1.3	3	Floral
11	28.4	2.3	3	Oily / Floral
12	29.8	1.0	3	Burnt / Medicinal / Smoky
Malt 1/10				
1	10.6	1.3	3	Vegetable / Sweet / Solventy
2	15.1	1.3	4	Floral / Burnt / Stale
3	22.0	2.7	3	Floral / Grassy / Sweet
4	23.3	2.3	3	Floral / Other
Malt 1/100				
1	10.6	1.7	3	Cereal / Nutty
2	22.0	1.8	4	Fruit / Sweet
3	23.3	1.7	3	Other / Floral / Fruit

Using the same approach as applied for the standard grain whisky, the chemical profiles of the odours remaining in the 1/100 fold dilution were further matched and evaluated for their identification. These odours gave relatively high odour activities in the unpeated malt whisky and were considered to be the potent aroma compounds for this matrix. The first compound began to elute at 10.6 min was 3-methyl-1-butanol, and its descriptors continually changed by dilution factor increased. The next one appeared at 22.0 min and was suggested as ethyl octanoate (m/z: 88/101, 85.2% match the reference library) by MS Spectral database (NTIS). The retention time and the related aroma characters of this compound were further confirmed by GC/MS-O by direct injection. Ethyl octanoate is a fatty acid which is a common by-product of fermentation, and has an aroma of wax and honey (Eggers et al. 2003), which agreed with the sensory

characters selected in this GC-O test (Grassy, Sweet and Fruit). The last odour began to elute at 23.3 min was identified as 2-phenylethanol (m/z 91/92, 76.4% match the reference library), which was again confirmed. This compound is a fermented aroma which has a sensory character of honey, spice, lilac and rose.

Identification of a marker compound for woody grain whisky

Table 3.32 shows the confirmed identified odours in the three dilutions of the woody grain whisky.

Table 3.32 – Confirmed identified aromas in the woody grain whisky.

No	Time	Average intensity	Number detecting aroma	Sensory character
Woody 1/1				
1	10.6	2.3	4	Cereal / Other / Solventy / Floral
2	12.3	1.7	3	Sweet / Grassy
3	15.1	1.3	4	Fruit/ Solventy / Smoky / Floral
4	22	2.7	3	Sweet / Floral
5	22.5	2.3	4	Smoky /Floral / Burnt/ Floral
6	23.3	2.0	3	Fruit / Grassy / Floral
7	24.2	2	3	Stale/ Medicinal/ Floral
8	26.2	1.0	3	Other / Sweet
9	27.3	2.3	3	Other / Fruit / Floral
11	32.3	3	4	Sweet
Woody 1/10				
1	22	2.0	4	Fruit / Sweet / Floral
2	23.3	2.5	4	Other / Floral / Sweet
3	24.2	1.5	4	Stale / Other / Nutty / Sweet
4	32.3	2.5	4	Sweet
Woody 1/100				
1	22.0	1.6	3	Sweet / Floral / Fruit
2	23.3	2.0	4	Floral / Spicy
3	24.2	2.0	4	Grassy / Floral / Nutty / Stale
4	32.3	3.0	4	Sweet

The four odours remaining in the 1/100 dilution had the highest odour activity (Table 3.32) in the woody grain GC-O AEDA test. As previously reported for the standard grain and the unpeated malt whiskies, the odours eluting at 22 min and 23.3 min were ethyl octanoate (m/z: 88/101) and 2-phenylethanol (m/z 91/92), respectively.

The odour that eluted at 24.2 min was recognised as *cis*-whisky lactone (*cis*-β-methyl-γ-octalactone) (m/z 99/87, 82.6% match the reference library). The retention time and aroma characters of its pure form were further tested and confirmed. This *cis*-isomer appears in oak woods, particularly at high levels in American white oak. The *cis*- and *trans*-isomers come from small amounts of lipids, oils, fats and waxes in the oak. The *cis*-isomer has more intense character than the *trans* and affects all beverages that are matured in new and used American oak casks (Jackson 1994).

The last odour eluting at 32.3 min was vanillin (m/z: 151/152, 87% match the reference library). The retention time and the aroma character for its pure form were tested and confirmed. Vanillin is one of the most important aroma compounds in whisky related to a whisky maturated character. From a sensory perspective vanillin imparts typical sweet aromas to whisky and has a relatively high odour activity (threshold: 0.17 mg/l from Table 2.4).

3.5.2.2 Quantitative analysis: Measurement of the concentrations of the marker compounds in each matrix type

Table 3.33 – Marker compound data comparison for 3 basic whisky matrices.

Marker compound	Retention time (min)	Sensory Threshold (mg/l)	Concentration in the whisky (mg/l)	Odour activity value (OAV)	Odour intensity (OI)*
Standard grain					
3-methyl-1-butanol	10.4	300	63	0.2	1.8
Unpeated malt					
3-methyl-1-butanol	10.6	300	1446	4.8	1.7
Ethyl octanoate	22.0	0.4	12.5	31.3	2
2-phenylethanol	23.3	10	21.8	2.2	1.7
Woody grain					
Ethyl octanoate	22.0	0.4	0.3	0.8	1.6
2-phenylethanol	23.3	10	1.6	0.2	2
cis-oak lactone	24.2	0.3	2.6	8.6	2
Vanillin	32.3	0.17	3.6	21.2	3

**Odour intensity data were obtained from GC-O measurements*

The concentrations of each of the potent odours identified in previous (GC-O AEDA) test were quantified to select the most potent aroma compounds for the three basic whisky matrices. The OAVs of these compounds were calculated based on the

compound's threshold and its concentration in the matrix. The resulting data is summarised in Table 3.33.

For the standard grain whisky the only odour compound detected in the 100-fold dilution was 3-methyl-1-butanol. However, as seen in Table 3.33, this compound had a low OAV of less than 1. The reason for its low OAV is because grain whisky matrix is naturally weak in its aroma congener concentration (see Chapter 3.3.1). Since 3-methyl-1-butanol has an OAV <1, and there are no other compounds present that are above threshold level, the standard grain whisky should theoretically have no detectable aroma. However, this demonstrates that odour units calculations may over-simplify aroma, as recent studies have provided more and more evidence that compounds at sub-threshold concentrations can influence overall aroma perception (Ryan et al. 2008; Saison et al. 2009).

3-Methyl-1-butanol, ethyl octanoate and 2-phenylenthanol are potential marker compounds for the unpeated malt whisky. 2-phenylethanol had a relatively low OAV due to its high threshold. Comparing the remaining two odours, ethyl octanoate showed the highest value within both OAV and OI (both from the GC-O and in the actual whisky matrix).

Ethyl octanoate, 2-phenylethanol, *cis*-oak lactone and vanillin were the four compounds found in the 1/100 dilution of the woody grain whisky. As described previously *cis*-oak lactone and vanillin are derived from oak wood and were hence thought to be the most suitable potential markers for woody grain whisky. In Table 3.33, vanillin had the highest OAV of 21.2 and OI = 3 (highest contribution in both GC-O and in the actual whisky matrix). Thus, vanillin was suggested to be the most appropriate marker compound to represent woody grain matrix.

GC-O AEDA was successfully applied to identify the most potent odour compounds in each whisky matrix. Marker compounds were selected as being the composition factors that have the highest impact on the AEDA-GC-O test and aroma contribution in each whisky matrix, which are for Grain matrix (3-methyl-1-butanol), Woody matrix (vanillin) and Malt matrix (ethyl octanoate). These three marker compounds were used in the next stage of the aroma interaction study.

3.5.2.3 Determination of the aroma interaction capacities of the marker compounds

The potential of the identified marker compounds to predict aroma interaction capacity (AIC) of the overall matrices was investigated (methodology explained in chapter 2.4.2). The concept behind this approach is that chemical compositions of the blend components significantly contribute to odour and aroma interaction in the matrix. The selected marker compounds were selected as being the composition factors that have the highest impact on the aroma contribution in each whisky matrix. It was hypothesised that the AIC of these individual marker compounds would give a good indication to the overall AIC of the matrix. All the marker compounds were prepared with equal aroma intensity matrices backgrounds (five times of its thresholds), then spiked with increase amounts stimulate for the thresholds measurement. The concept and methodology was similar to the threshold technique used in Chapter 3.1.1.2, in that it again used thresholds as a measure of aroma interaction capacity (AIC). The AIC of each maker compound, at their five times OAV concentrations, is shown in Table 3.34.

Table 3.34 – Marker compound aroma interaction capacity with their five time OVA concentration.

Matrix	Marker compound	AIC (v/v %) (peated malt masked)
Grain	3-methyl-1-butanol	2.05 %
Woody	vanillin	1.60 %
Malt	ethyl octanoate	2.85 %

Table 3.34 showed that peaty character was masked by all three marker compounds preset matrix. The degree of aroma interaction capacity varied from compound to compound, Ethyl octanoate showed the highest degree of aroma interaction with peaty character, while vanillin had the least effect. This is an interesting phenomenon suggesting that under the same equal aroma intensity background intensity (in this case five OAV) different aroma compounds exhibit different aroma interaction capacity to peaty character. This agrees with the previous studies (Saison et al. 2009; Sterckx et al. 2011) that compounds can exhibit their aroma characteristic independently. As a result, it was concluded that each marker has its own aroma characteristics and has a different capability impact on peaty aroma perception through aroma interaction. This conclusion led to the next stage in the study where the relationships between the AICs of the marker compounds and that of the host matrices were explored.

3.5.2.4 Predictive modelling based on the AICs of marker compounds

The concentration, threshold, and marker's AIC were all obtained from previous study. Then the marker's predicted AIC can be calculated based on this information, the model was built as following steps in Table 3.35. The relationship between the observed and predicted AICs was then compared. The resulting data are shown in Table 3.35.

Table 3.35 – Observed AIC versus calculated AIC (based on maker compounds).

	Matrices	**Standard Grain**	**Woody Grain**	**Unpeated Malt**
	Markers	3-Methyl-1-Butanol	Vanillin	Ethyl Octanoate
1	**Concentration (mg/l)**	63	3.6	12.5
	Threshold (mg/l)	300	0.2	0.4
	Concentration ÷ Threshold = odour activity value (OAV)			
2	**OAV**	0.2	21.2	31.3
	Individual marker's AIC per five OAV	2.05	1.60	2.85
	OAV × Marker's AIC per OAV = Predicted (marker based) AIC			
3	**Predicted AIC**	0.1% (v/v)	6.8% (v/v)	17.8% (v/v)
	Observed AIC	2.2% (v/v)	4.1% (v/v)	6.1% (v/v)
	10% (v/v Caol Ila) ÷ Predicted AIC = predicted peaty intensity			
4	**Predicted peaty intensity**	100	1.5	0.6
	Observed peaty intensity	1.4	0.7	0.7

It is clearly shown in Table 3.35 (4) that the prediction of the peaty strength, the maker based model failed with both order and value magnitude, as the woody has an AIC more than two times higher than malt, the observed woody and malt almost same value, and the grain predicted strength (100) far larger than the actual observation value.

It was also shown that the two sets of results were very different in numerical terms for their aroma interaction capacity (AIC) as well. For example, the predicted AIC for the standard grain whisky was 0.1% (v/v) peated malt, compared to 2.2% (v/v) for the observed AIC. The predicted AIC results for standard grain and unpeated malt are both unrealistic in actual sensory terms. Such a small difference is unlikely to be detected in

sensory terms. Conversely, for the unpeated malt the predicted AIC value was very large (17.8% (v/v) peated). Above 10% (v/v) of peated malt concentration, peaty character should be easily detected in real blending practice.

From both predicted peaty strength and AIC's, the values were either too high or too low, thus indicating the models lack predictive power in this particular study. The relationship is a sigmoidal curve (Figure 3.22). According to the sigmoidal curve, it would be assumed that if the compound was present at an OAV <1 then it would not have any aroma interaction capacity. Only once the OAV reached 1 it would have the potential to mask peat character. However, this aroma interaction capacity would not continue to increase linearly, rather would reach a maximum response after which higher levels would have no further sensory impact (Figure 3.22).

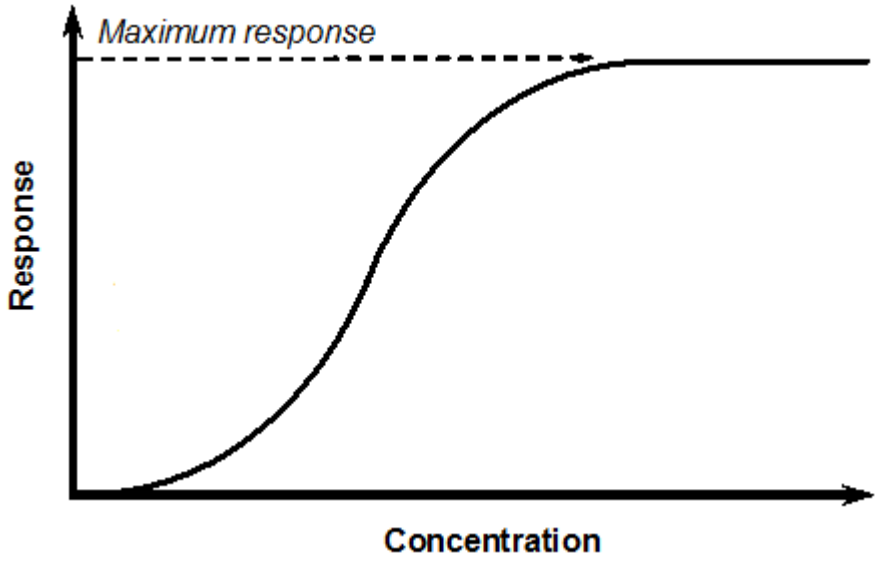


Figure 3.22 – Sensory and stimulant sigmoidal curve relationship.

Usually the relationship between the sensory perception and concentration obeys to Steven's Law (Moskowitz, 1977). However, in this case the sample size was too small to allow the Steven's Law exponent to be calculated.

3.5.2.5 Summary

In conclusion, the predictions were far off-scale of actual observation and delivery the wrong ranking with the prediction peaty strength. The failure of this marker based model provides information that should be considered for future chemical based models. Firstly, and most importantly, a whisky matrix contains hundreds of aroma active congeners, which in combination give the complex overall aroma sensation of the

whisky (Meilgaard 1975; Olsson 1994; Saison et al. 2009). The single marker compound is very limited in its ability for representation of the whole matrix. Secondly, this study used a limited sample size to determine the feasibility of the concept. To achieve a better and more reliable correlation, the sample size and the number of experiments should be maximized. Finally, the identified marker compounds were the common aroma compounds existing in most of the whisky types and categories. Thus, these compounds would also have contributed to aroma interactions in the other whisky types, which also need considered.

With a few exceptions, it remains challenging to unequivocally attribute a given aroma perception to a specific chemical entity. As we have shown here the intensity of aromas has been influenced by the matrix in which they were assessed. In summary, Scotch whisky aroma is complex matrix with no single congener, or cluster of congeners, responsible for its whole aroma character. There is still a need for an accurate and reliable method to quantify the sensory perception purely based on analytical measures. Although the hypothesis of using marker compounds to develop a prediction model of aroma interaction capacity for the whisky matrix was not successful, it was still a useful attempt to understand aroma interactions using a different approach.

3.5.3 Prediction model based on peated malt threshold

The prediction based on the phenol threshold approach was found more successful than the marker based prediction. Through the validation test Chapter 3.5.1.5, it was found that the thresholds of the phenols in the different matrices do have the ability to reflect the aroma interactions happening in the blend. However, it also had clear drawback, as the thresholds of all eight phenols had to be measured in each component of the matrix, which was extremely time and labour consuming and not practical in industrial applications. Also, although the model was based on all eight phenols routinely measured, it still did not fully represent peaty aroma perceptions.

To overcome these drawbacks, a peated malt threshold approach has been tried based on the thresholds and odour unit principle. In this study, the single compound stimulant was replaced by individual peated malt (Caol Ila), and the odour unit of peaty aroma calculated using Equation 1(Chapter 1.5.2.3).

In order to create a blended whisky there are many different types of whisky that can be used such as peated malt, unpeated malt, standard and woody grain. Therefore, the intensity of peaty aroma in a particular blend is calculated as a basis of odour unit (O_n) and its related matrix volume percentage. For a particular whisky type the volume percentage of each of the component whiskies can vary. A typical blend generally comprises of a large number of different whisky components, each making up a small volume percentage in the total blend. Taking this proposition to a logical extreme gives a blend with a peaty calculated strength shown in Equation 4.

In this equation, C is the aroma concentration, AIC is the aroma interaction capacity and V is the volume percentage of an individual blend components. Based on Equation 4 it is possible to calculate the peaty response based on threshold and volume of the each blending components used. As mentioned previously, the sensory perception and concentration is not a linear relationship but a sigmoidal curve (Figure 3.22). And it would assume that once the Odour unit is above 1, a blend component would have the potential to interact with (mask) peat character. However, this masking capacity would not continue to increase linearly, rather would reach a maximum response after which higher levels would have no further impact, i.e. when peaty character can no longer be detected. The relationship between the sensory perception and concentration obeys Steven's Law (Moskowitz, 1977), which is used to relate the sensory perception to any changes in a physical magnitude, and the concentration is expressed as Equation 5:

$$Sensory = K \times Concentration^{n} \qquad \textbf{Eq. 5}$$

Linear variation in concentration, normally used in sensory experiments, could lead to exaggerated variation in sensory perception, and the sensory performance can be optimized by Steven's Law exponent (0.3 – 0.8 for odorants) (Friedrich and Acree 1998). In this study, by combining the sensory threshold approach and Steven's Law, the overall peaty aroma strength in a blend whisky can be expressed as Equation 6:

$$Observed\,Strength = K \times Calculated\,Strength^{n} \qquad \textbf{Eq. 6}$$

In Chapters 3.1 - 3.4, aroma interaction capacities were determined for a range of different whiskies. These were used to establish the relationship between overall peaty sensory strength and matrix aroma interaction capacity.

In the first series of blending experiments, simple two component blends were studied. These comprised of 10% v/v of the stimulant (Peated malt: Caol Ila) mixed with 90% v/v of either standard grain, woody grain or unpeated malt. The concentration of peated malt was also included as part of overall blending matrix background, since its sensory and chemical properties are closer to malt whisky. The blending combinations for these studies can be expressed using Equation 7:

$$Calculated\ peaty\ intensity = \left(\left(\frac{10\%}{FIC}\right)_x \times 90\%\right) + \left(\left(\frac{10\%}{6.12\%}\right)_p \times 10\%\right) \quad \textbf{Eq. 7}$$

In this equation, background matrix (x) volume is 90% v/v and stimulant volume (p) is 10% v/v. Since the stimulant also considered in this equation as a malt whisky, the unpeated malt matrix AIC (6.12% (v/v)) was used here. Therefore, in order to calculate the peaty intensity based on different whisky blending blocks, the only parameters are required are the matrix aroma interaction capacities (AICs). These were already determined in Chapters 3.1-3.4, but are summarised again in Table 3.36 alongside the calculated strengths based on Equation 7. Observed peaty intensity, as scored by the sensory panel, is also shown in Table 3.36.

Table 3.36 – Aroma interaction capacity and calculated and observed peaty intensity for different two components blend matrices.

Chapter	Matrices	Threshold (AIC)	Calculated peaty intensity	Observed peaty intensity
3.1	Standard grain	2.2	4.3	1.1
	Woody grain	4.1	2.3	0.6
	Unpeated malt	6.1	1.6	0.5
3.2	Linkwood	6.1	1.6	0.7
	Clynelish	6.9	1.5	0.6
	Knockando	6.8	1.5	0.8
	Dailuaine	7.2	1.4	0.7
	Glendullan	6.7	1.5	0.7
	Cardhu	6.5	1.5	0.7
	Blair Athol	6.3	1.6	0.7
	Benrinnes	5.9	1.7	0.7
3.3	Cameron Bridge	2.9	3.3	1.0
	Invergordon	2.2	4.3	0.9
	Port Dundas	2.3	4.0	1.0
	Girvan	2.6	3.7	1.0
3.4	woody2	3.4	2.8	0.9
	woody4	3.6	2.7	0.7
	woody7	4.0	2.4	0.7
	woody9	4.7	2.1	0.5
	woody 12	4.8	2.0	0.6

Note: all the sample were test based on same peaty concentration (10% Caol Ila v/v)

It was noted that every component with a calculated intensity less than 2 was a malt whisky, while all of the grain whiskies had calculated intensity more than 2. The malt whiskies showed very consistent results and small variation. On the other hand, the standard and woody grain whiskies showed greater variation in both observed and predicted results.

Based on the data in Table 3.36 it is possible to plot a graph between calculated and observed peaty intensity to obtain the Steven's Law coefficient K and exponent *n* (Figure 3.23).

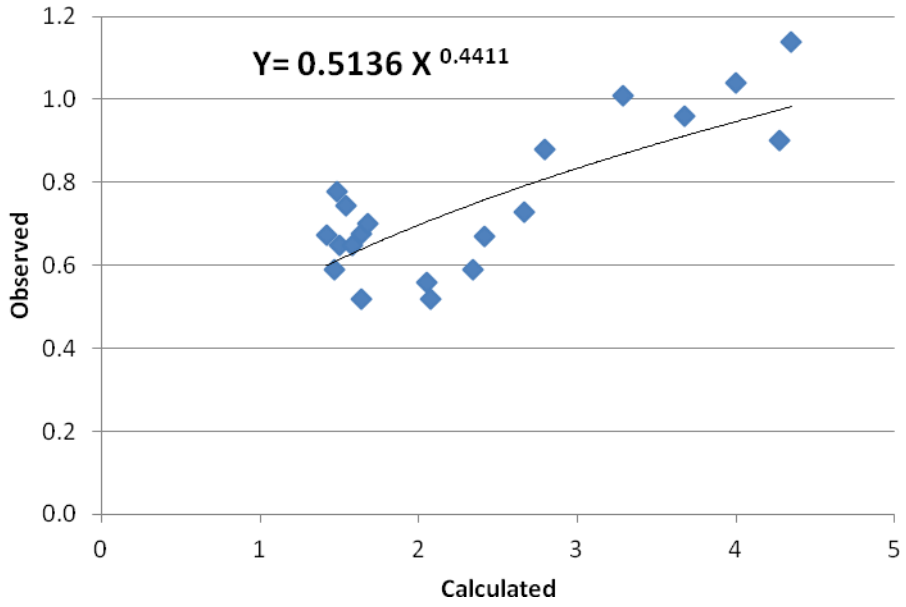


Figure 3.23 – Observed versus calculated peaty intensity

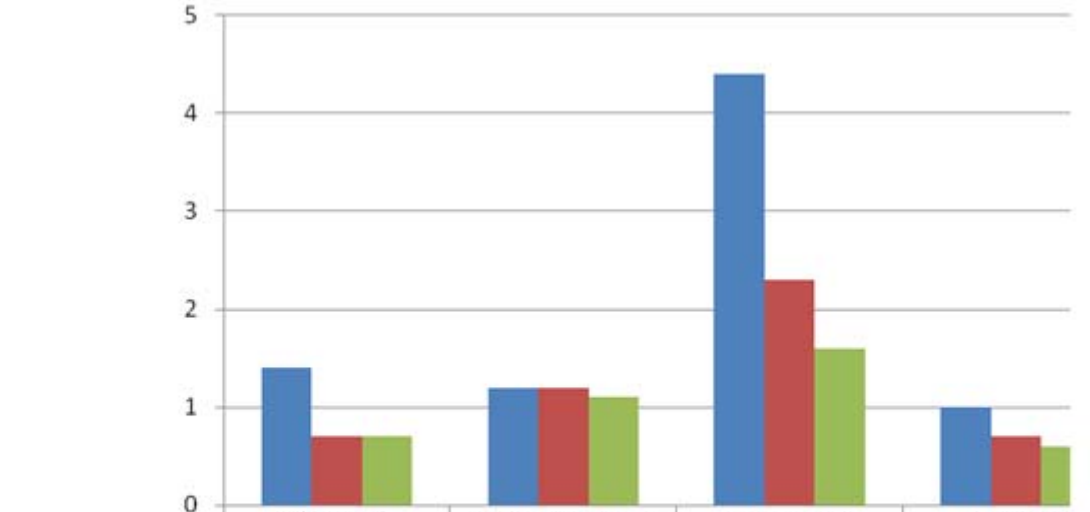
As being shown in Figure 3.23, the peaty strength prediction model was then completed as Equation 8:

$$Observed\,Strength = 0.5136 \times Calculated\,Strength^{0.4411} \qquad \textbf{Eq. 8}$$

By apply this equation, it is possible to estimate the peaty intensity of the blends, which were made by combining four basic whiskies (Table 2.5). To further examine the accuracy of the prediction model, more blend samples were created to evaluate the model accuracy in following Chapters (Chapters 3.5.3.1 - 3.5.3.5). Series sets of blends were created to further test and verify the model's accuracy. These blends contained single, double and triple matrix components with different levels of peated malt addition.

3.5.3.1 Evaluation of single component matrices

Three samples were prepared using a single blending element (standard grain, woody grain or unpeated malt) with the addition of 10% (v/v) peated malt (Caol Ila). Results obtained from various approaches are compared in Figure 3.24. The observed peaty intensity (panel scores), levels of total phenols, calculated peaty intensity based on Equation 7, and predicted peaty intensity based on Equation 8 are included in Figure 3.24.



	Observed Peaty Intensity	Analytical (Total phenol)	Calculated Peaty Intensity	Predicted Peaty Intensity
Standard grain	1.4	1.2	4.4	1
Woody grain	0.7	1.2	2.3	0.7
Unpeated malt	0.7	1.1	1.6	0.6

Figure 3.24 – Comparison of analytical data, observed, calculated and predicted peaty intensity for whisky blend containing single blending element.

As shown in Figure 3.24 the observed peaty intensities were in the order Grain > Woody = Malt, with the standard grain having double the peaty intense of the woody grain and unpeated malt matrices. It was also seen in Figure 3.24 that the traditional analytical phenols method failed to distinguish between three blends, with similar results being obtained for all three samples. As described previously, this analytical method is insufficient to explain the perceived peaty intensity in blends because the effects of the aroma interactions are not considered. Calculated peaty intensity, obtained from Equation 8, was capable of distinguishing the differences between three samples. However, the results were not in the same magnitude as the observed sensory scores.

Finally the predicted peaty intensity, based on Equation 8, showed a similar pattern to the observed results (Grain > Woody ≈ Malt), and the results were very close to the observed values in magnitude for the woody grain (observed 0.7, predicted 0.7) and the

unpeated malt (observed 0.7, predicted 0.6). The standard grain showed more variation (observed 1.4, predicted 1.0). As a result the following Chapter focuses on the predicted intensity.

Overall, this threshold based model successfully differentiates and predicts differences in peaty intensity in a single component matrix and gives a relatively accurate prediction value. The next step was to evaluate the model using samples containing two blending components.

3.5.3.2 Evaluation of double component matrices

Here 10% (v/v) peated malt was added to matrices containing two blending elements. Three combinations were evaluated: standard grain vs. unpeated malt, standard grain vs. woody grain and woody grain vs. unpeated malt mixtures. Series of blends were prepared with different ratios of these components. Previous experiments (Chapter 3.1) showed that standard grain had lower masking capacity comparing to the unpeated malt and woody grain. Therefore, when the standard grain was blended with other two matrices, the standard grain was considered as a diluting factor.

Standard grain vs. unpeated malt mixtures

Five samples were prepared containing different proportions of standard grain (G) and unpeated malt (M): 100G (100% grain (v/v)), 75G/25M (75% grain and 25% malt (v/v)), 50G/50M (50% grain and 50% malt (v/v)), 25G/75M (25% grain and 75% malt (v/v)), and 100M (100% malt (v/v)). 10% (v/v) peated malt was added to these blending matrices and the resulting five samples compared. The observed and predicted peaty intensities are shown in Table 3.37.

Table 3.37 – Observed and predicted peaty intensity for standard grain and unpeated malt blends.

Grain/Malt blends	Peaty Intensity	
	Predicted	Observed
100G	0.98	1.03
75G25M	0.91	0.85
50G50M	0.83	0.77
25G75M	0.74	0.65
100M	0.64	0.60

As shown in Table 3.37, with a decreasing amount of grain in the blend, both the observed and predicted peaty intensities correspondingly decreased (Table 3.37), showing that peaty intensity is negatively correlated to malt content. The predicted and observed data showed clear correlation and similar numerical values, both of which indicated at least some predictive power of the model to the real sensory observations.

Another interesting finding is that the sensory value changed between first and second half of the experiments. If we consider the 50G50M as the middle point of this test, the observed sensory difference between 100G to 50G50M is 0.26, where the difference between 100M to 50G50M is 0.17. In other words, the sensory response change in the first half (100G to 50G50M) test is greater than the second half experiments (50G50M to 100M).

If we consider the Grain/Malt blending experiments as the dilution test, the results can be compared with the previous malt and grain studies (Chapter 3.3). As shown in Figure 3.12 and Figure 3.16, the grain based blends gave observed peaty intensities that varied between 0.90-1.04, where malt based blends varied between 0.59-0.78. In this Grain/Malt blending test the observed peaty intensities were varied between 0.6-1.03 (Table 3.37), which almost covered both malt and grain based study data range. The first three data points 100M (0.60), 25G75M (0.65) and 50G50M (0.77) all fell within the previous eight malt study variation range, but the 50G50M is on the edge of this data range (Table 3.20). When the dilution continues to 75G25M (0.85) the peaty intensity clearly falls between the data range of the malt and grain sets. Finally, as expected, 100G falls into the grain data range (Table 3.16).

In this Grain/Malt blending test, when 50-75% (v/v) of malt been replaced by grain whisky, it aromas interaction capacity just start fall out the previous eight malt based measurement (Table 3.20). However, if we compare analytically between 50G50M or 75G25M with all other previous malt studies (Table 3.17), the aroma congener of these two samples are far below the average of the eight malt samples. This finding again confirms the previous conclusion that aroma congener concentration and matrix aroma interaction capacity are not well correlated, and using analytical method to predict the peaty intensity outcomes in Malt and Grain matrices has its limitations.

The correlation between the observed and predicted peaty intensity was explored to test

the accuracy of the prediction model.

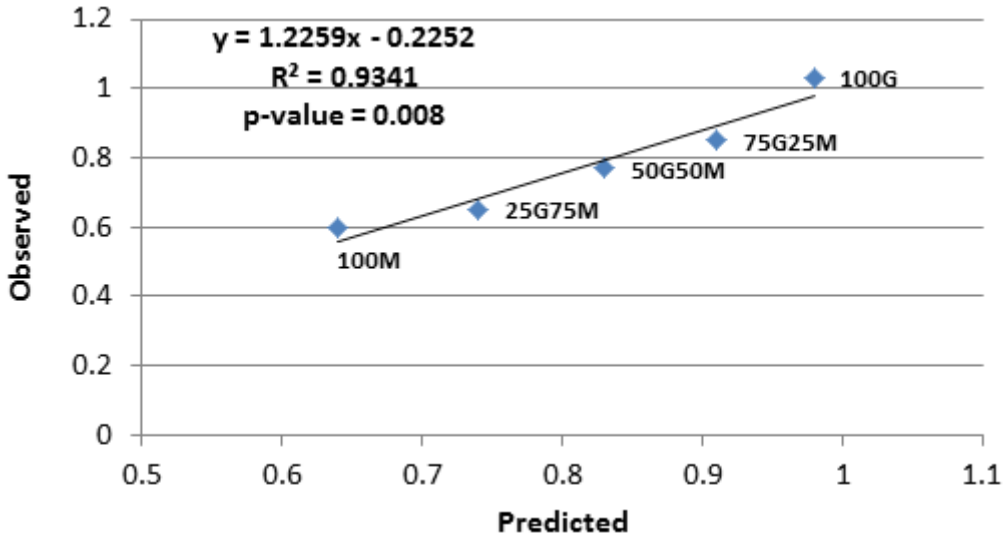


Figure 3.25 – Regression plot of the observed against the predicted peaty intensity for Malt/Grain blends.

According the Figure 3.25, it showed good prediction accuracy based on the gradient and intercept. From Figure 3.25, it was found that gradient of 1.2259 was larger than one with intercept was -0.2252. This regression parameter indicates that the model overestimates peaty intensity in high malt models and underestimates it in high grain models. The regression the R-squared ($R^2 = 0.9341$) and p-value (0.008) show a good correlation between prediction value to observed results, which indicates that the threshold based model provides an acceptable predicting in these two component blends.

Standard grain vs. woody grain mixtures

A similar experiment was carried out to examine standard grain/woody grain blends. The predicted peaty intensity was calculated based on the Equation 6. The observed and predicted peaty intensities for the five blends are shown in Table 3.38.

Table 3.38 – Observed and predicted peaty intensity for standard grain and woody grain blends.

Standard/woody blends	Peaty Intensity	
	Predicted	Observed
100G	0.98	1.10
75G25W	0.93	0.94
50G50W	0.87	0.76
25G75W	0.81	0.75
100W	0.75	0.61

Again a similar phenomenon was observed. If we consider the 50G50W as the middle point of this test, the observed sensory difference between 100G to 50G50W is 0.34, where the difference between 100M to 50G50W is 0.15. In other words, the sensory response change between 100G to 50G50W was more than double the change between 50G50W to 100W. With an increasing level of woody grain in the blend, the peaty intensity detected by the sensory panellists showed a noticeable decrease. The predicted results also decreased. The observed data showed a bigger range 100G(1.1) - 100W(0.6) = 0.5 than the predicted 100G(1) - 100W (0.75) = 0.25. Statistical analysis was applied to determine the correlation between the observed and predicted peaty intensities. Result is shown in Figure 3.26.

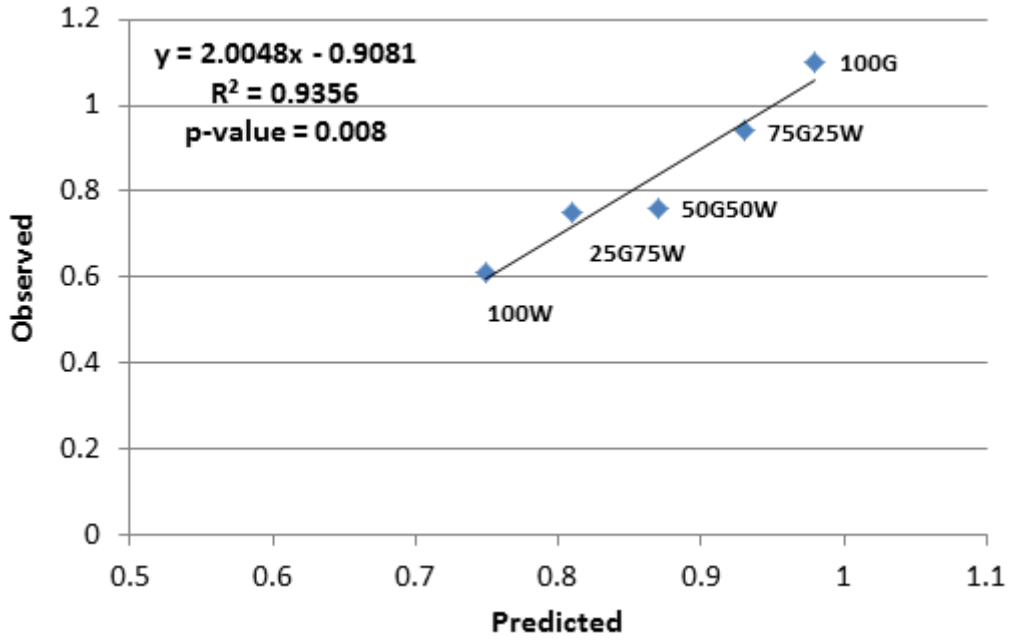


Figure 3.26 – Regression plot of the observed against the predicted peaty intensity for the standard/ woody grain blends.

A significant correlation (p-value = 0.008) was found between the observed and the predicted peaty intensity with an R-squared = 0.9356. The gradient was 2.0048 and intercept is -0.9081. Again the threshold based model gave a relatively accurate means of predicting actual sensory response, though the accuracy was not quite as high as that observed in the previous grain/ malt test. There is also the physical boundary in this regression model as in realistic you cannot get woodier than 100% (v/v) or more grain than 100% (v/v). If only consider the standard/woody grain blending experiments as a dilution test, the analytical data and observed peaty intensity can be compared with the previous woody based AIC study (Chapter 3.4). The observed peaty intensity and total maturation derived congeners are compared in Table 3.39.

Table 3.39 – Comparison of observed peaty intensity and levels of maturation derived congeners.

Grain/Woody blending test			Woody Study		
Sample	**Observed Peaty intensity**	**Total maturation derived congeners**	**Sample**	**Observed Peaty intensity**	**Total maturation derived congeners**
100G	1.10	14.2	woody2	0.88	33.5
75G25W	0.94	20.4	woody4	0.73	40.1
50G50W	0.76	26.6	woody7	0.67	37.2
25G75W	0.75	32.7	woody9	0.52	44.1
100W	0.61	38.9	woody 12	0.56	44.4

As expected all of the standard/woody grain blend samples, with the exception of the 100W, contain less maturation derived congeners than the previous sample set of grain whiskies with different degree of woody character. They also had higher scores for peaty intensity compared to the wood study samples. In other words, they contained less maturation derived congener and had less AIC, which consequently give higher peaty intensity. This experiment once again proved that the maturation derived congeners have an acceptable relationship with woody character and aroma interaction capacity.

The standard/woody grain blending test can treat as the expansion of the previous woody study (Chapter 3.4), since these blends (100G – 25G75W) simulate the situation of grain that has been matured less than two years. Consequently, two sets of results can be combined to give a wide range of maturation derived congeners and AICs, from very light (G100) to very heavy (woody 12). The individual maturation derived congener was compared with the observed peaty intensities to further investigate the correlation between these and AICs. Results of these linear regression analyses are shown in Table 3.40, with the exception of coniferaldehyde, sinapaldehyde and 5-HMF, the maturation-derived congeners showed significant correlations with aroma interaction capacity. Vanillin, scopoletin and total maturation derived congeners showed particularly high correlations. Vanillin has shown consistent high correlation with AIC in the previous woody study (Chapter 3.4), GC-O study (Chapter 3.6) and this two component blending study. This demonstrates that analytical markers can be used to indicate woody character and it related AIC, with vanillin and total maturation derived congener being particularly good measures.

Table 3.40 – Results of linear regression analysis between maturation derived congeners and AICs.

Woody congeners	R^2	p-value
Gallic Acid	0.479	0.027*
Ellagic Acid	0.727	0.002*
Coniferaldehyde	0.099	0.377
Vanillin	0.958	< 0.0001*
Vanillic Acid	0.612	0.007*
Sinapaldehyde	0.025	0.666
Syringaldehyde	0.615	0.007*
Syringic Acid	0.581	0.010*
Scopoletin	0.836	0.0002*
5-HMF	0.023	0.678
Total	0.965	< 0.0001*

**p-values < 0.05 showing significant correlations between the chemical compounds and the AIC*

Woody grain vs. unpeated malt mixtures

Woody grain and unpeated malt blends were also tested and the results are shown in Table 3.41.

Table 3.41 – Observed and predicted peaty intensity for woody grain and unpeated malt blends.

Woody/Malt blends	Peaty Intensity	
	Predicted	Observed
100W	0.75	0.93
75W25M	0.72	0.60
50W50M	0.70	0.60
25W75M	0.67	0.48
100M	0.64	0.45

Again the change in the sensory response over the first half of the tests (100W-50W50M = 0.33) was more than double that observed in the second half (50W50M-100M = 0.15). A similar phenomenon was also observed in the previous standard grain/malt and standard/woody grain tests, which suggested the introduction of low levels of heavy components gives relatively large sensory impacts (AIC changes) than further increased in heavy components. This phenomenon could be because at the beginning of the test, the matrix background is relatively simple, so an increased proportion of a heavy matrix will have more impact on the AIC, whereas in the second half of the tests, the matrices are already heavier than the first half, and a further in heaviness cannot give much more impact on AIC.

The matrices used in this part of the study, namely the unpeated malt and woody grain, are both relatively complex. As the malt content is increased, the woody content decreases, and it would be expected that one type of aroma interaction would be replaced by the other. The predicted peaty intensity ranges in the Grain/Malt study were (100W – 100M = 0.34) and the Grain/Woody (100G – 100W= 0.23), while in this Woody/Malt study the range was much small (100G – 100W= 0.11). However, in the observed date the differences were much more similar, Woody/Malt test (100W – 100M = 0.46), Grain/Malt test (100G – 100M = 0.43) and Grain/Woody test (100G – 100W= 0.49). These data range differences between predicted and observed results highlight a difference between the sensory panel data and the prediction model. This may be caused by many factors such as the fact that individual samples may be influenced by other samples present or that the predictive model coefficient and exponent were obtained from wider data sets. The relationship between the observed and predicted peaty intensities was explored by linear regression analysis (Figure 3.27).

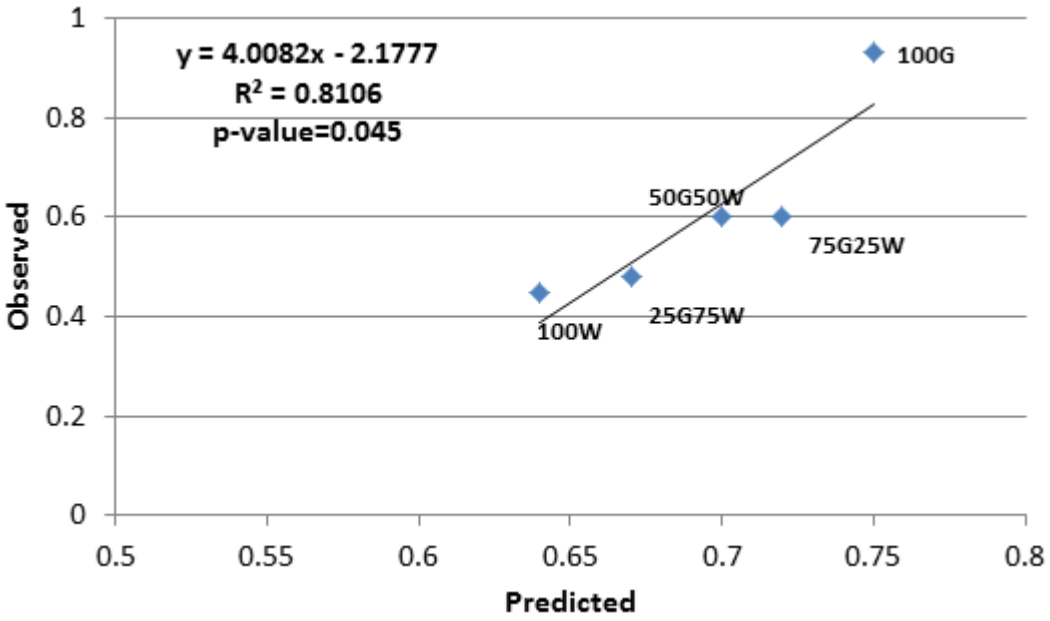


Figure 3.27 – Regression plot of the observed against the predicted peaty intensity for the malt/ woody grain blends.

The regression analysis showed a significant correlation (p-value = 0.045) between the observed and predicted peaty intensity with an R-squared = 0.8106, gradient = 4.0 and intercept = - 2.18. Clearly the accuracy of this prediction was not as good as the predictions observed in the previous two component blend tests.

Malt and Woody are both heavy matrices, with quite small differences in their aroma interaction capacities (Table 3.36), This may have caused difficulties for threshold-based predictive model to identify the different aroma interactions between the malt and woody grain component. Highly aromaful matrices also introduce more interference (noise) to the sensory test, which will also impact on the accuracy of the prediction. Overall, the predictive model has shown good capacity to predict peaty intensity when one matrix is being diluted by a neutral component. Mixing two heavy matrices reduces the accuracy of the prediction.

3.5.3.3 Evaluation of triple component matrices

In this Chapter the tests were carried out to evaluate the model accuracy on the blends with three blending components, included combinations of standard grain, woody grain and unpeated malt. Two sets of experiments were designed and each set was run in four sensory sessions (one session a day). In each session, the level of one component (woody or malt) was kept as constant and other two varied (Table 3.42). The peated malt (Caol Ila) was added as a constant 10% (v/v) level.

Table 3.42 – Experimental plan for triple component blending test.

Day (session)	constant components	Constant components	Variable components
Series one			
1 [W10]	10% (v/v) peated malt	10% (v/v) woody	Standard grain↓ Unpeated malt↑
2 [W20]	10% (v/v) peated malt	20% (v/v) woody	
3 [W30]	10% (v/v) peated malt	30% (v/v) woody	
4 [W40]	10% (v/v) peated malt	40% (v/v) woody	
Series two			
1 [M10]	10% (v/v) peated malt	10% (v/v) malt	Standard grain↓ Woody grain↑
2 [M20]	10% (v/v) peated malt	20% (v/v) malt	
3 [M30]	10% (v/v) peated malt	30% (v/v) malt	
4 [M40]	10% (v/v) peated malt	40% (v/v) malt	

The observed and predicted peaty intensities for each session are shown in Table 3.43. For better comparison, all of the data were tested by linear regression to assess the correlation between the predicted and observed values (Figure 3.28).

Table 3.43 – Observed and predicted peaty intensities. Regression R-squared and p-value obtained from regression test at 5% significance level. The labels indicate the blend composition (not including the peated malt volume).

Series One

W10	Observed	Predicted	**W20**	Observed	Predicted	**W30**	Observed	Predicted	**W40**	Observed	Predicted
W10G90M0	1.15	0.96	W20G80M0	1.11	0.94	W30G70M0	0.94	0.92	W40G60M0	0.97	0.90
W10G80M10	1.01	0.93	W20G70M10	1.01	0.91	W30G60M10	0.90	0.89	W40G50M10	0.88	0.87
W10G70M20	0.97	0.90	W20G60M20	0.94	0.88	W30G50M20	0.82	0.86	W40G40M20	0.75	0.83
W10G60M30	0.62	0.87	W20G50M30	0.88	0.85	W30G40M30	0.75	0.83	W40G30M30	0.72	0.80
W10G50M40	0.49	0.84	W20G40M40	0.91	0.82	W30G30M40	0.79	0.79	W40G20M40	0.76	0.76
W10G40M50	0.51	0.81	W20G30M50	0.82	0.78	W30G20M50	0.58	0.75	W40G10M50	0.76	0.73
R^2	0.92		R^2	0.89		R^2	0.85		R^2	0.32	
p-value	0.002		p-value	0.005		p-value	0.008		p-value	0.059	

Series Two

M10	Observed	Predicted	**M20**	Observed	Predicted	**M30**	Observed	Predicted	**M40**	Observed	Predicted
M10G90W0	1.21	0.95	M20G80W0	0.90	0.93	M30G70W0	0.84	0.90	M40G60W0	0.83	0.86
M10G80W10	1.08	0.93	M20G70W10	0.79	0.90	M30G60W10	0.80	0.87	M40G50W10	0.82	0.84
M10G70W20	1.01	0.91	M20G60W20	0.63	0.88	M30G50W20	0.79	0.85	M40G40W20	0.61	0.82
M10G60W30	0.68	0.89	M20G50W30	0.53	0.86	M30G40W30	0.64	0.83	M40G30W30	0.65	0.79
M10G50W40	0.52	0.87	M20G40W40	0.57	0.83	M30G30W40	0.59	0.80	M40G20W40	0.61	0.76
M10G40W50	0.55	0.84	M20G30W50	0.46	0.81	M30G20W50	0.61	0.77	M40G10W50	0.72	0.74
R^2	0.93		R^2	0.90		R^2	0.88		R^2	0.37	
p-value	0.002		p-value	0.003		p-value	0.006		p-value	0.218	

Overall the range of the predicted values is much smaller than the range in the observed peaty intensities. In Table 3.43, the complexity of the blend matrices consistently increases from top to bottom (malt% (v/v) increase) and left to right (woody% (v/v) increase). Within each session, as the complexity increase from top to bottom, the observed peaty intensity decreased. The predicted results followed the same pattern, decreasing as the complexity increases from top to bottom and left to right. However the accuracy of these predictions was relatively poor for both vertical and horizontal comparisons. Comparing each session horizontally, the correlation between the predicted and observed data gradually reduced as complexity increased.

The poor correlations with complex blends are mainly due to the variation in the observed sensory results. For example, the observation results showed a random rather than a regular decrease tendency in the Series two M40 test, where the woody content increased and malt remained the same. As the whole became more heavy and complex, the observed results appeared to be more random. Linear regression was carried out to explore the relationships between the observed and predict data across the full set of samples (Figure 3.28).

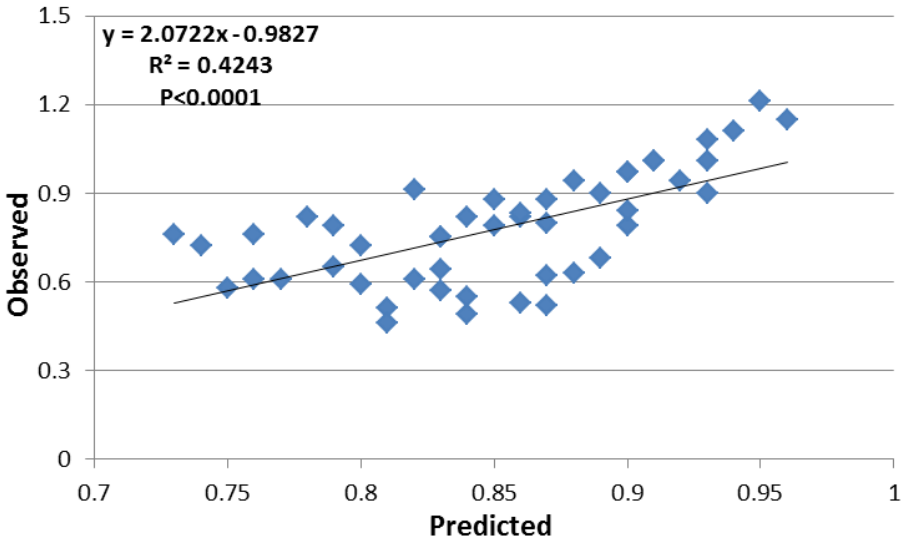


Figure 3.28 – Regression plot of the observed against the predicted peaty intensity for the triple component blends.

As being shown in Figure 3.28, the observed and predicted peaty intensity scores were significantly correlated ($p<0.0001$), with R-squared value (0.4243). The gradient (2.07) and intercept (0.98) values show that the accuracy of the prediction for these triple component blends was not particularly good. Overall, based on the results of the double and triple

component tests, the model is not good at predicting peaty intensity in more complex mixtures, such as blends containing malt and woody grain whiskies.

3.5.3.4 Validation using different peated malt concentrations

In previous Chapters 3.5.3.1-3.5.3.3 different combination of matrix backgrounds were tested for their ability to interact with peaty aroma, and the threshold based peaty aroma prediction model was validated. The results showed that this new prediction model could be a potential way to predict the peaty aroma intensity when matrix AIC need considered. However, this conclusion is based on studies that used the same level of peated malt (10% (v/v) Caol Ila). To further test the threshold based model, an additional experiment was tested with different levels of peated malt addition, similarly as previous phenol based validation test Chapter 3.5.1.3. Samples were evaluated for the peaty intensity by three methods (observed peaty intensity, predicted peaty intensity and traditional analytical measurement of total phenols) and the results are shown in Figure 3.29.

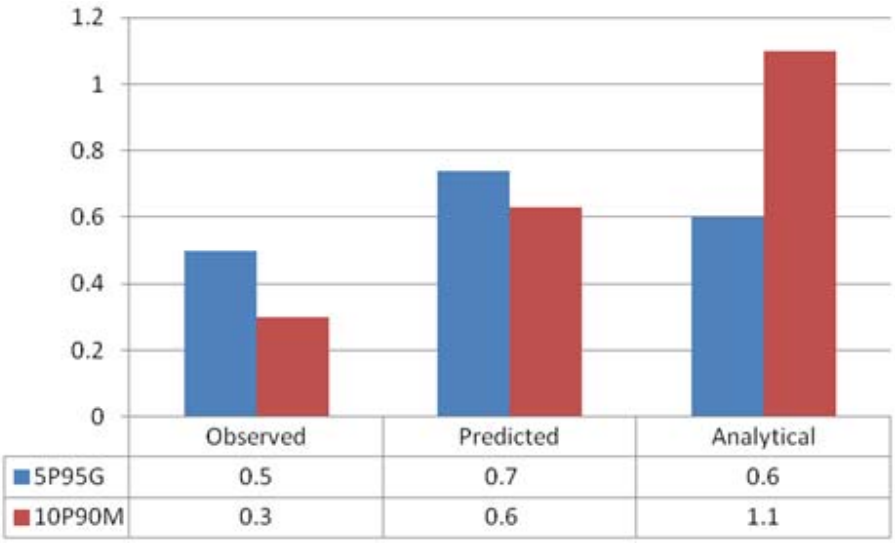


Figure 3.29 – Observed and predicted peaty intensity and analytical data for blends containing different concentrations of peated malt.

The traditional analytical based method did not give data that corresponded to observed peaty intensity. Based on the phenol levels the 5P95G should have about half of the peaty intensity of the 10P90M. However, in reality the 5P95G had double the peaty response, again showing strong evidence of the presence of the aroma interactions during blending. The predictive model rated the two samples in the correct order, but the accuracy of the

production was poor. It was again quite clear that total phenol concentration (analytical data) fails to explain differences in sensory perceptions. The total odour units, calculated based on the phenol thresholds, was able to predict the intensity relationship between 5P95G and 10P90G, although the prediction values are inaccurate.

Compared with the phenol threshold based model, the peated malt threshold based model gave roughly the same quality prediction, as the prediction ratio between 5P95G and 10P90G in both prediction models are all approximately 1.2.
(Simpson et al. 1974)

3.5.3.5 Accuracy and precision of the prediction model

In this Chapter 3.5, the prediction model has been examined with single, double, triple and different stimulus concentration blends. By comparing the predicted and observed results, it can be concluded that the prediction results are generally quite close to the observed results. The accuracy of the prediction is especially accurate when matrix is relatively simple, e.g. only one component (Chapter 3.5.3.1) or containing a simple and a heavy component (Chapter 3.5.3.2), whereas when the matrix becomes more complicated the difference between the predicted and observed results become much bigger. However, the observations made here may not simply be due to a prediction error. To further explore this difference, the variation in the observed sensory data was examined below.

Evaluation of sensory variation in the single component blend test

By comparision between panels of the sensory data (scaling test) obtained in the single component blend test were tested by repeating the analysis three times in different days (Test 1-3). Single component blends (100G, 100M and 100W) were also tests in the double component blend test (Chapter 3.5.3.2), which gave two more sets of test results (Test 4-5). The individual results for each session, means and relative standard deviations are shown in Table 3.44.

Table 3.44 – Scaling test repeatability.

Matrices	Standard Grain	Woody grain	Unpeated Malt
Test 1	1.41	0.72	0.69
Test 2	1.14	0.59	0.52
Test 3	1.68	0.89	0.84
Test 4	1.03	0.61	0.60
Test 5	1.10	0.93	0.45
Average	1.27	0.75	0.62
RSD	21%	21%	25%

The relative standard deviation (RSD) for repeatability was about 20-25% for all three samples. This variation in the sensory data may be due to the fact that no control sample was used in the scaling test. Panellists can use different part of the line-scale in each experiment, and the panel composition can vary of different days. Finally the setup of the test was different between Test 1-3 and Test 4 and 5, in Test 1-3 there only three blends been present (Grain, Woody and Malt) but Test 4-5 have five samples and done in different days. Clearly, the Test 1-3 more stable and repeatable than 4-5, as the experiments for test 4-5 was not design for testing the AICs different between the above three blends.

Evaluation of sensory variation in the triple component blend test

The triple components test was carried on in two series, which involved two-thirds of the sensory experiments being repeat tested. These repeat results, means and relative standard deviation are shown in Table 3.45.

Comparison of the sensory results obtained in Series 1 and Series 2 showed quite big differences, with RSDs as high as 36%. The average RSD across the data sets was around 20%, which was similar to the variation observed in the single component Test. One factor that influences the sensory data is the experimental design, namely the number and types of samples presented within a particular sensory session. A minor difference between two samples may influenced by the other samples in the set. For example, the panel might detect a difference between Sample A and B if there are no other samples in test. However, if a third sample (C), is present which has a more distinct difference, then this may make A and B appear more similar in sensory terms. Also the order in which samples are presented can affect subsequent sensitivities.

Table 3.45 – Scaling test repeatability

Sample code	Series 1	Series 2	Average	RSD
W10G50M40	0.49	0.82	0.66	36%
W10G60M30	0.62	0.8	0.71	18%
W10G70M20	0.97	0.79	0.88	14%
W10G80M10	1.01	1.08	1.05	5%
W20G40M40	0.91	0.61	0.76	28%
W20G50M30	0.88	0.79	0.84	8%
W20G60M20	0.94	0.63	0.79	28%
W20G70M10	1.01	1.01	1.01	0%
W30G30M40	0.79	0.65	0.72	14%
W30G40M30	0.75	0.64	0.70	11%
W30G50M20	0.53	0.82	0.68	30%
W30G60M10	0.68	0.9	0.79	20%
W40G20M40	0.76	0.61	0.69	15%
W40G30M30	0.59	0.72	0.66	14%
W40G40M20	0.57	0.75	0.66	19%
W40G50M10	0.52	0.88	0.70	36%

Based on the scaling data from the single and triple components test, it can be that the variation in the sensory results, at around the 20% RSD level. Generally speaking an RSD of around 20-30% is considered acceptable for sensory analyses (Lundahl and McDaniel 1988; Blank 2002). However, this variation causes difficulty for the predictive modelling. The predictive model works on a different principle, as the model based on components % differences that would not always to be detected by the sensory panel.

It was clear from the double and triple components validation that as the blend matrix becomes more complex or when whisky with a heavier aroma is used, the peaty character differences between samples become smaller. If there are no major differences between samples, then secondary differences become more apparent. Human sensations may adapted to the change and enlarge the minor differences (Borg 1982; Green et al. 1996; Lawless et al. 2000). However, the predictive model does not enlarge or narrow the sensory response gap between samples in the same way as human sensation. Models will quantity the difference between samples based the data provided, which will not be influenced in the same way as the sensory experiments. Therefore, the threshold based model can often predict the right sensory response order but poorly predict the actual values.

The value differences obtained between the observed (sensory) and predicted (calculated) results can be explained as differences in accuracy and precision. Precision is how repeatable the analysis is, whereas accuracy is how close the result are to the actual value. In this case the prediction model is precise, but may not be accurate, whereas the observed sensory results are accurate but not precise. Overall, sensory tests always have an inherent degree of variation, whereas the predictions based on composition will be free from this error. Therefore minor changes predicted by the model may not give rise to noticeable sensory differences

Summary

Both the peated malt and phenols models provide a better prediction than traditional phenols measurement, as the aroma interaction was pre-measured for each matrix's aroma interaction capacity. These two methods were similar in their predictive ability, but the peat based method is relatively easier to conduct with actual blending practice as only peated malt threshold need to pre-measured rather than all eight phenols.

CHAPTER 4. CONCLUSION

It is very important for the whisky manufacturer and the master blender to know the intensity of peaty character in their blend. The primary finding of this research is that the peaty character of a blend is not only related to the amount of peated whisky present. The goal of this project was to try to understand the mechanism of peaty aroma behaviour in blended whisky and find possible approaches to predict peaty character. This was carried out by first exploring the major factors that influence the peaty aroma in blended whisky, then to build prediction models based on these factors.

4.1 Factors influencing perceived peaty aroma intensity

A series of tests were carried out to determine the factors that influence peaty aroma (Chapter 3.1). This work was based on an aroma and chemical profile comparison of four basic whisky types. From the results it was confirmed that the amount of peated malt added and its peating level (phenol content) have a direct influence on overall peaty aroma intensity. As expected, if more peated malt is added into a blend then the more peaty it becomes.

However, it was also found in Chapter 3.1.2, that when the same amount of peated malt was added (blended) in four different matrices (ethanol, standard grain whisky, woody grain whisky and unpeated malt whisky) the sensory panel observed a clear difference in peaty aroma intensity, despite the phenol headspace concentrations being almost identical in the four different blends. The findings in this experiment suggest that traditional instrumental methods (namely phenols analysis) may not always provide an accurate prediction of peaty intensity. This is because the overall peaty intensity in a blend is not only influenced by the peated malt content, but is also affected by other blending components and the blend complexity, with matrices such as unpeated malt and woody grain having a stronger effects on reducing the perception of peaty aroma. These effects must be taken into account during whisky blend design, as the same amount of peated malt used in different blends will not necessarily give the same degree of peaty character. Physiological aroma interactions were found to be the main factor responsible. So, further

work was carried out to understand and model these interactions.

4.2 Measuring aroma interaction

Two sensory approaches (Chapter 3.1 - 3.4) were developed to measure the aroma interaction capacity of different matrices; 1) scaling and 2) threshold measurement. The threshold method gave good quantification, but was costly in terms of time and labour, so was only applied to the original blending component whiskies. In contrast, the scaling method was less time consuming so was used to measure the relative relationships between all the samples.

4.3 Differences in aroma interaction capacity among whiskies

To further study peaty aroma interaction, more whisky samples were tested (Chapter 3.2 – 3.4), namely four standard grain, five woody grain and eight unpeated malt whiskies. The results of these studies revealed interesting findings:

Firstly it was confirmed that different categories of whiskies interact with peaty character differently. Compared with malt matrices, standard and woody grain whiskies have less aroma interaction capacity. However, within the same category the aroma interaction capacities (AICs) were very similar. This suggests to the blender, that the Grain/Malt ratio is most important, while the individual distillery sources of these grains or malts have minimal influence. It was also noticed that no individual or clusters of chemical were identified that can represent the grain or malt matrix's AIC. There are chemicals that can be used as markers to distinguish different between grain and malt matrices, but these do not provided information on sensory relationships.

In the woody grain study, woody aromas generally increased with maturation time and there is a corresponding increase in wood derived congeners. AIC also increased with higher woody character, and vanillin, one of the main woods derived congeners, was found have a good correlation with both overall woody intensity and aroma interaction capacity.

4.4 Predictive models

Models were developed to predict peaty character. The principle of these models was based on two pieces of information, firstly the peated malt content in the blend and then the influence of the aroma interaction caused by individual components in the blend matrix. Hence, quantifying the influence of different whiskies on peaty aroma was critical to the model, with AICs being determined by threshold measurement.

Three prediction models were developed and tested, all based on a core Equation 1. This equation converts the stimulant concentration into a perception outcome, while also taking the AIC into account.

The three sets of predictions were calculated from models based on

1. Prediction model based on phenols thresholds (Chapter 3.5.1)
2. Prediction model based on potent aroma marker compounds (Chapter 3.5.2)
3. Prediction model based on peated malt threshold (Chapter 3.5.3)

4.4.1 Model based on phenols thresholds

Since phenols have been found to give a good correlation with peaty aroma in simple two component blends, the initial thought for this study was try to predict the peaty aroma intensity in more complex blends through phenol analysis. This prediction was based on the aroma impact of blend components on the sensory thresholds of the 8 major phenols, the assumption for this approach being that the impact on the individual phenols will be similar to the impact on overall peaty aroma.

The prediction based on the phenol threshold approach was found to be more accurate than that obtained when directly comparing sensory perceptions outcomes simply with the levels of phenols present, as demonstrated in Table 3.26Table 3.27. Through this validation test, it was shown that the thresholds of the phenols in the different matrices do have the ability to reflect the aroma interactions happening in the blend. Also, this phenols-based approach had the benefit of being semi-analytical, with phenol concentrations (stimulant) being

measured instrumentally. However, it also had clear disadvantages, namely that the thresholds of all eight phenols had to be measured in each component of the matrix, which was extremely time and labour consuming and not practical in industrial applications. Also, although the model was based on all eight phenols routinely measured, it still did not fully represent peaty aroma perceptions.

4.4.2 Model based on potent aroma marker compounds

In the first model the focus was on measuring the influence of the whole matrix on the stimulant (phenols). In this second model experiments were designed to identify individual marker compounds, these being the most potent aroma compounds in each of the matrix components (unpeated malt, standard grain and woody grain). Each marker's aroma interaction capacity was then determined, by combining its concentration with its impact on peaty aroma. This in turn was used to predict peaty aroma.

The most potent aroma compounds (markers), identified through a GC-O study, were 3-methyl-1-butanol for the standard grain whisky, ethyl octanoate for the unpeated malt whisky and vanillin for the woody grain whisky. This agreed with earlier findings in Chapters 3.3 and 3.4 where 3-methyl-1-butanol was found to be a good marker for distinguishing grain and malt whiskies, while vanillin was identified as a good marker for woody character.

The three basic matrices were tested to validate this marker compound approach. According to the results showing in Table 3.35, the predicted AIC did not correspond to the observed, in terms of magnitude values and right ranking. This suggests, the marker AICs have no direct linkage with the overall matrix aroma interactions capacity.

Scotch whisky aroma is complex with hundreds of aroma congeners present. Using a single potent aroma to represent the overall aroma interaction proved to be inadequate for predicting the peaty aroma intensity in blends. Moreover, if one compound cannot represent the whole matrix aroma behaviour, then simply increasing the number of the compounds is unlikely to make this marker compounds approach representative of actual matrix aroma interaction behaviour.

4.4.3 Model based on peated malt threshold

A final model was developed that related back to the more successful phenols threshold approach. Although the previous approach had been relatively successful in predicting peaty aroma outcome it was time and labour intensive, and even combining all eight major phenols still did not fully represent the whole peaty characteristics. Also, the prediction results were not of the same magnitude as the observed peaty score. To overcome these disadvantages, a final model was developed based on measuring aroma interactions between matrix components and peated malt. Using the peated malt directly as the stimulant overcame two disadvantages. Firstly only one threshold (AIC) needed to be determined for each whisky matrix and secondly using peated malt itself was the best way to represent actual peaty aroma. Secondly, Steven's law was introduced into the model to explain the relationship between perceived magnitude and concentration of a sensory stimulus. The AICs of more whisky matrices had been measured (Chapters 3.3 – 3.4), which gave a sample group big enough to work out Steven's Law coefficient and exponent (Chapter 3.5.1). Through Steven's Law, the relationship between AIC and overall peaty aroma intensity was established.

Overall, the prediction models for this study may not be accurate for the actual sensory data, but are precise enough to be used for blending practice. However, it is still not a fully instrumental prediction, depending instead on data being required on the sensory interactions between the stimulant and the matrix components.

4.5 Implications for the whisky industry and blending practice

In terms of the meaning of this study for industry, it has provided lots of valuable data that is useful for blending practice.

- Traditional instrumental measures of phenols levels cannot predict peaty intensity in blends as aroma interactions between the peated whisky and the other blend components also need to be considered.
- Aroma interaction with the matrix background can be as important a factor as the peated malt addition level (Chapter 3.5.1.3).

- Aroma interaction capacity is highly influenced by the whisky background's overall aroma intensity. In other words, the stronger of the aroma of the other components of the blend the bigger the aroma interaction capacity.
- Standard grain whisky has a lower aroma interaction capacity compared to unpeated malt and woody grain whiskies.
- AIC increases with maturation
- In terms of the peaty aroma intensity, the differences in AICs between unpeated malt distilleries is not as important as the overall percentage malt content.
- In terms of the peaty aroma intensity, peated malt aroma differences between distilleries is not as important as the overall peaty intensity.
- Peaty aroma intensity can be predicted from a model based on interactions between the stimulant (peated malt) and the individual blend components.
- peated malt content, malt/grain ratio and age of the whisky can be the three key factors to effect the peaty aroma intensity in a particular whisky blend in blending practice.

CHAPTER 5. FURTHER WORK

The observations from this work have indicated the complexity of the peaty aroma interactions the blended whiskies, with many factors that can potentially alter and affect peaty aroma intensity. There is still a requirement to increase the amount of data, both sensory and analytical, and to further explore peaty aroma interactions in a wider range of whiskies in order to improve the model for predicting the peaty intensity in Scotch whisky blends. Suggestions for further studies follow:

1. The final model was mainly based on the addition of one particular peated malt at a fixed addition level. Although, this work has shown, the profile of phenolics at least for peaty spirits is similar, just varying in absolute concentration. The Future work should still be carried out to verify test this model using blends made with different type of peated malts at a range of addition levels.

2. According to the results of the validation test (Chapter 3.5.2), it was found that when the matrix make up was simple (single and most double components blends), the prediction results were relatively accurate. However, when the matrix make up became more complex the variation between the predicted and observed results became bigger, as the results obtained in the Woody/Malt double blending test and the triple component tests showed. Differences were found between the observed and predicted results, but as discussed previously this can explain as the differences of accuracy and precision. Sensory tests by their nature are relatively noisy, where the predictions based on composition will be free from this error. Therefore, minor changes predicted by the model may not give rise to noticeable sensory differences, which another factor should take into account to improve the model to overcome the sensory noise influence.

3. In this study, the maturation-derived compounds and their related masking capacity were identified by combining the analysis (analytical, sensory and statistical

analysis). However, in reality the woody aroma and its related masking capacity are undoubtedly more complicated, due to the development of stronger maturation characters every year and with different types of casks available for whisky maturation. Therefore, more work should be carried out to investigate the relationship between the various aspects of maturation and their related aroma interaction capacity. Also it would be worthwhile to investigate whether the peaty character aroma interactions reach a maximum at a certain maturation time or look at the degree of time difference likely to give a detectable sensory impact.

4. The thresholds and "odour unit" was found to be a very useful index for aroma research. The work here examined the threshold difference between different whisky matrices, which were used to quantify and predict peaty intensity (odour units). This research was based on a trained expert sensory panel. However, sensory response can vary from panel to panel. For commercial blending it may be more appropriate to use consumer panels to more accurately represent the perceptions of the target consumer.

5. This study only involved one aspect of aroma interactions occurring during blending. Individual whiskies are all very complex in aroma terms. Blending them together could trigger a tremendous amount of multi-aroma interactions, not just in terms of peaty aroma but also all the other whisky aroma attributes. For a better understanding of overall blend aroma, other aroma attribute interactions should also be investigated.

6. An additional area that might be interesting to explore is numerical methods to increase the accuracy of sensory data in future related aroma interaction studies.

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